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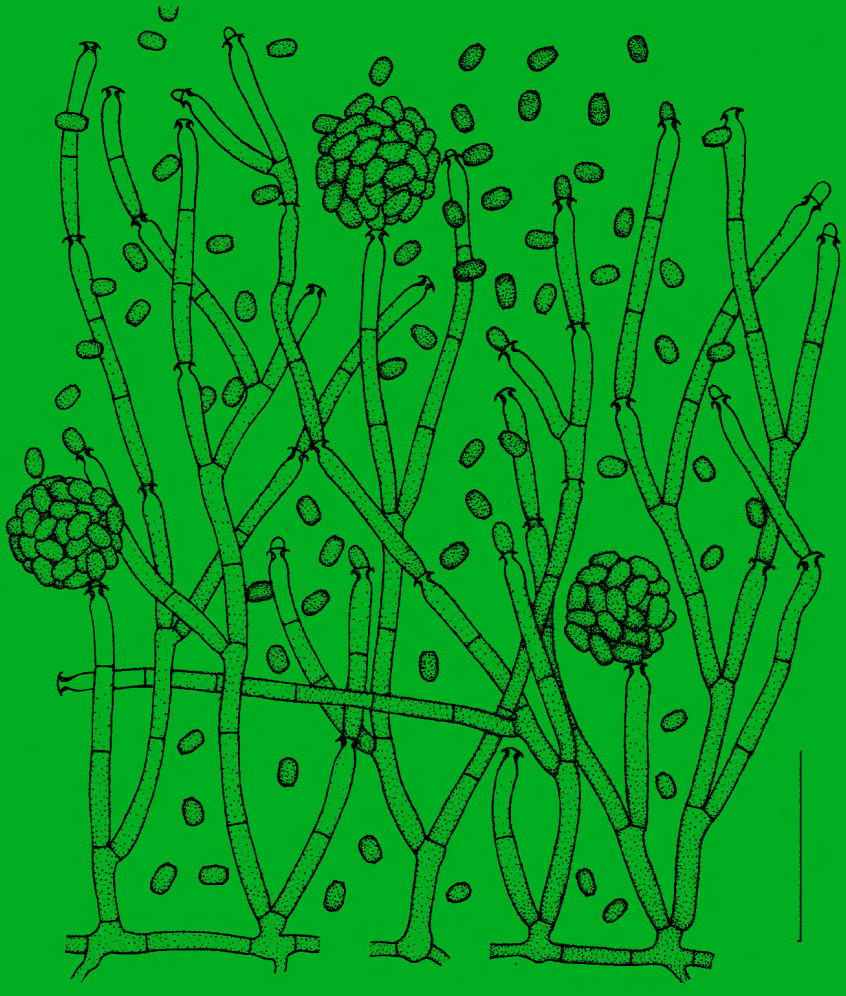

Phialophora nielamuensis sp. nov.
(Wu & Zhang— FIG. 2, p. 253)
YUE-MING WU, *artist*

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From the Editor-in-Chief

Welcome to Mycotaxon Online! When we wrote, "Print is dead. Long live the pixel!" a few months ago, we somehow hoped that with the end of press and postal delays our first volume would just leap onto the website all by itself. Alas — the quantity of editorial work remains the same, and a late Mycotaxon 114 led to an equally late Mycotaxon 115, while we adjusted our workflow for on-line publication and revised author instructions for what we all hope is the very last time. Although the first online volume *is* late, our taxonomic insights are now accompanied by free living color. Enjoy!

Cover section — We have combined the former editorial 'front matter' (masthead information, table of contents, publication dates) and 'back matter' (reviewer list, editorial letter, errata, submission protocols) into one free access PDF, opening now with an original drawing chosen from among current papers and ending with our belief that good taxonomy is vital to everyone. (Special thanks to Noni Korf Vidal for the refurbished logo!). The author index has disappeared—no longer needed in the Google era. The 101 new names proposed or typified in our first online edition stand in a separate handy, free-access PDF at the end of the volume.

"Weblists" are regional mycobiotas — When we first set up the Mycotaxon regional annotated species list webpage, we called the posted PDFs 'weblists' to convey the idea that these papers were available for free download from the Internet. However, that term does not fully describe what these works encompass — nomenclature, geography, ecology, substrata, and (often) brief descriptions or illustrations. Only when revising how to submit such works for posting on our non-journal website, did I realize that these treatments are, in fact, 'mycobiotas' (a term used instead of 'mycoflora' to emphasize that fungi are not plants).

Obviously, Mycotaxon still publishes mycobiotas presenting original and complete taxonomic research in its online journal. For that reason, we will continue to refer to the less comprehensive distributional compilations as 'weblists.' Pages 534–536 announce six additions to our website and cites authors, titles, and abstracts for species reported from Central America (Nicaragua, Panama), South America (Brazil), Europe (Greece, Italy), and the Mid-East (Turkey).

Open access — Our cover section, book reviews, weblist abstracts, and nomenclatural pages are freely available to anyone on the Internet, and we encourage authors to offer their papers for immediate release by paying a $20/page open access fee. Mycotaxon 115 offers open access articles by Cabrera & Vobis (53–63), Raut & al. (227–238), D.-W. Li (239–250), Schmidt & Schlosser (287–301), J. He & al. (303–310), S.A. Rodrigues & al. (335–344), Y.-Z. Wang (365–368), S.-H. He & H.-J. Li (375–382), Oktay & al. (425–433), and McCune (485–494). Special thanks are due them for showing that Mycotaxon is well on its way to becoming a fully open access journal.

Warm regards,
Lorelei Norvell,
Mycotaxon *Editor-in-Chief*
28 April 2011

Errata from previous volumes

Volume 114

p. iv, line 31	*for:* Gregario Delgado 231	*read:* Gregorio Delgado 231
p 342,page number	*for:* no page number	*read:* 342
p. 375, header	*for:* Minimelanolocus chimonanthi	read: *Minimelanolocus chimonanthi*
p. 377, line 2	*for:* 377–393	*read:* 377–392
481, abstract, line 6	for: *lividina*	read: *fascicularia*
p. 483, line 14	for: *lividina* Hjortstam	read: *fascicularia* (Rick) Burds. & Nakasone
p. 517, line 31	*for:* 377–393	*read:* 377–392

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A new species of *Ophiocordyceps* (*Clavicipitales, Ascomycota*) from southwestern China

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Abstract — A new species of caterpillar fungus, *Ophiocordyceps laojunshanensis*, is described based on specimens collected from southwestern China. This species is characterized by slender stromata, sparse perithecia, and the unique habitat of growing in mosses. Its habitat, gross morphology, and microscopic features are illustrated and relationships to similar species discussed.

Key words *Cordyceps, Ophiocordyceps sinensis, Ophiocordycipitaceae,* taxonomy

Introduction

The genus *Cordyceps* includes over 400 species, of which 120 species have been reported in China (Liang 2007). Although the genus was formerly classified in the *Clavicipitaceae*, based on recent results of the multi-gene phylogeny of *Cordyceps* sensu lato, the taxonomy of both *Cordyceps* and the *Clavicipitaceae* has been revised.

A new family *Ophiocordycipitaceae* has been proposed based on the genus *Ophiocordyceps* Petch, which has been emended by Sung et al. (2007). *Ophiocordyceps* is now characterized by stromata that are darkly (or rarely brightly coloured) pigmented, tough, fibrous, pliant to wiry and rarely fleshy, often with aperithecial apices or lateral pads, perithecia that are superficial to completely immersed and arranged ordinally or obliquely (Sung et al. 2007).

In the past three years, the authors undertook numerous mycological explorations to mountainous Northern Yunnan, where they collected many specimens of caterpillar fungi that grow among mosses and have morphological features that do not correspond to any previously described taxa. One new species, *Ophiocordyceps laojunshanensis,* is described and illustrated in this paper.

Materials & methods

Field notes were taken for gross morphology of fresh ascomata and habitat. Detailed macroscopic features were examined under a stereomicroscope (Carl Zeiss Discovery 12). Microscopic examination of ascomata and measurements of microscopic structures were made from freehand sections under a microscope (Olympus 520). The sections were mounted in 5% aqueous KOH and then Cotton–blue lactophenol. The holotype of *Ophiocordyceps* laojunshanensis was deposited in the Cryptogamic Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences (KUN–HKAS 57066). Isolates from fresh ascomata of *O. laojunshanensis* were maintained on potato dextrose agar with 1% peptone (PPDA) using techniques described by Liu et al (1989), all isolates were kept in Key Laboratory of Tropical Forest Ecology, Xishuangbannan Tropical Botanical Garden, Chinese Academy of Sciences (No. lao1–lao20).

Taxonomy

Ophiocordyceps laojunshanensis J.Y. Chen, Y.Q. Cao & D.R. Yang, **sp. nov.** Fig. 1

MycoBank MB 517206

Stromatibus clavatis, singularibus, 47.0 93.0 mm longis, 1.0 3.9 mm crassis. Capitulis cylindricis, 8.5 17.0 mm longis, prophyro brunneis vel atro brunneis, apis acerosis sterilibus 3.5 8.0 mm longis. Perithecia globosa, 200 300 × 200 350 µm; Stipitibus, 25.0 72.0 mm longis; Asci clavuli, cylindraci, 165 275 × 11.5 14.5 µm. Ascosporis hyalinis, filiformis, multiseptatis, 130 250 × 5.0 6.0 µm.

Type: China: Yunnan Province, Lijiang Naxi Autonomous Prefecture, Laojunshan mountain, 26°37'33.76N 99°42'58.07E, alt. 3974 m; under *Rhododendron mariae* Hance; associated with *Oncophorus wahlenbergii* Brid. and *Pleurozium schreberi* (Brid.) Mitt., 8 May 2009, Ji yue Chen 09032.(**Holotype:** KUN HKAS 57066).

Etymology: The epithet refers to the type locality, Laojunshan Mountain

Stromata clavate, slender, simple, rarely 2 or 3 from host head, rarely 1 from host head and another from the terminal region, 47.0–93.0 mm long, 1.0–3.9 mm in diam. Ascogenous portion almost 1/5 to 1/3 of the stromata length, cylindrical, 8.5–17.0 mm long, purplish to dark brown. Apex sterile acuminate, 3.5–8.0 mm long. Perithecia, globoid, 200–300 × 200–350 µm. arranged loosely in irregular lateral cushions. Stipe 25.0–72.0 mm long. Asci clavate, 165.0–275.0 × 11.5–14.5 µm. Ascospores hyaline, filiform, septate, 130.0–250.0 × 5.0–6.0 µm (Fig. 1a–f).

Colonies (F ig. 1g–l) grew very slowly, only up to 6–10 mm in diameter after 2 months on PPDA at 16°C. They were white at first, then became brown–yellow or black brown, and sparse white mycelia emerged 3 month later, with conidiophore and conidia, reverse blackish brown. Conidiophore, hyaline, branching or non–branching; conidiogenous cell phialidic, hyaline, with verrucose, acerate, 15–39(–50) µm long; Conidia, hyaline, long elliptic, 6.0–13.5 × 3.0–4.0 µm, one or two encased in a mucus drop.

Host: Larvae of *Thitarodes yunnanensis* Nielsen et al. (*Hepialus yunnanensis* Yang et al.)

Fig 1. *Ophiocordyceps laojunshanensis* – a. habitat; b. fresh fruiting body; c. dry fruiting bodies; d. ascogenous portion, bar = 2 mm; e. ascus with ascospore, bar = 20 µm; f. ascus tip, bar = 20 µm; g h. culture (bar = 5 mm): g. surface, h. reverse; i. colonies, bar = 20 mm; j. conidiophore and conidia, bar = 20 µm; k. conidiophore (detail), bar = 25 µm; l. conidia (detail), bar = 10 µm.

Additional specimens examined: CHINA: yunnan province, Lijiang Naxi Autonomous Prefecture, Laojunshan mountain, 26°37'03.98N 99°42'18.02E to 26°37'82.71N 99°42'97.86E, alt. 3874-4075 m. Under *Abies delavayi* Franch and *Rhododendron mariae* Hance. Associated with *Oncophorus wahlenbergii* Brid., *Dicranum muehlenbeckii* Bruch & Schimp. or *Pleurozium schreberi* (Brid.) Mitt., 8 May 2009, Ji yue Chen 09033 09035.

Discussion: *Ophiocordyceps laojunshanensis* is characterized by slender stromata, sparse perithecia, and its unique habitat of growing among mosses. It strongly resembles the most precious caterpillar fungus, *O. sinensis* (Berk.) G.H. Sung et al., and is often sold together with that fungus. The two species could be living in the same host larvae, except that *O. laojunshanensis* is found most often growing among mosses, while *O. sinensis* appears to grow more frequently in grasslands. Morphologically, *O. laojunshanensis* differs from *O. sinensis* by having a slimmer stroma with a shorter ascogenous portion and relatively sparse irregular perithecia compared to the close ordered perithecia found in *O. sinensis*.

Acknowledgments

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Rediscovery of *Microporellus iguazuensis* in southern Brazil

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Abstract — *Microporellus iguazuensis* is redescribed from the southern Atlantic rainforest of Brazil 23 years after its original description. The species is characterized by stipitate basidiomata, smooth ellipsoid basidiospores, simple-septate generative hyphae, dextrinoid skeletal hyphae, and a root-parasite habit. In the present work this noteworthy polypore is described, illustrated and its taxonomy discussed.

Key words — *Polyporaceae*, *Basidiomycota*, neotropical fungi

Introduction

The genus *Microporellus* Murrill comprises about 19 accepted species worldwide with a predominantly tropical distribution (Kirk et al. 2008). *Microporellus* was originally described as a segregate from the also tropical genus *Microporus* P. Beauv., which presents a trimitic hyphal system and coralloid dichophytic elements along the dissepiments (Ryvarden & Johansen 1980), features lacking in *Microporellus*. The taxonomic concept of the genus (Decock 2007) includes pileate species with a dimitic hyphal system and, mostly, dextrinoid skeletal hyphae and subglobose spores. The following species are known from Brazil: *M. brasiliensis* Ryvarden & Decock (Decock & Ryvarden 2002), *M. dealbatus* (Berk. & M.A. Curtis) Murrill (Loguercio-Leite & Wright 1991), *M. obovatus* (Jungh.) Ryvarden (Rajchenberg & Meijer 1990), and *M. terrestris* (Gibertoni & Ryvarden) Decock (Decock 2007).

In the present paper, we report the occurrence of a rare and poorly known *Microporellus* species that was collected during polypore surveys in the Atlantic rainforest. This ecosystem is located throughout most of the Brazilian Atlantic coast from the northeast to the south, covering the lower eastern slopes of the

mountain ranges (Morellato & Haddad 2000). It experiences a warm climate with abundant rainfall and, as one of the world's most endangered forests, is considered a hotspot worthy of conservation and research (Mittermeier et al. 2005).

Materials & methods

Basidiomes were collected between March 2007 and March 2009 in the northeast region of Rio Grande do Sul State, southern Brazil. Macro- and microscopical analyses followed usual polypore protocols (Núñez & Ryvarden 2001). Microscopic examinations were made from freehand sections mounted in a drop of 5% KOH solution and 1% phloxine solution; amyloid or dextrinoid reactions were observed in Melzer's reagent. The cyanophilous reaction was observed in cotton blue. Abbreviations follow Coelho (2005): Dm = diameter mean, Lm × Wm = means of length and width, Q = range of length/width ratios, Qm = length/width mean, and n = x/y, with x = number of measurements from y = number of specimens. Color names and codes follow Kornerup & Wanscher (1978). All the specimens are preserved at the ICN Herbarium (UFRGS).

Taxonomy

Microporellus iguazuensis Rajchenb., Mycotaxon 28: 111, 1987. FIGS 1–8

SPECIMENS EXAMINED: BRAZIL. Rio Grande do Sul State, Dom Pedro de Alcântara, RPPN Mata do Professor Baptista, 27.III.2007, leg. M.A. Reck 035/07 (ICN 139893); Porto Alegre, Refúgio da vida Silvestre Morro Santana, 30.III.2007, leg. M.C. Westphalen 003/07 (ICN 154057); leg. M.C. Westphalen 006/07 (ICN154060); 13.III.2009, leg. M.A.Reck 004/09 (ICN154263).

Basidiomes annual, lateral to eccentrically stipitate, coriaceous and flexible when fresh, corky and rigid upon drying. Pileus up to 6.0 × 4.0 cm, flabellate-spathulate, with fusions between one or more parts of them, margin regular, some incurved upon drying; upper surface glabrous to slightly velutinate, zonate in shades of orange red (8.B7) and pastel red (8.A4), becoming pale upon drying, dull red (8.C3) with reddish brown zones (9.E6); stipe erect, lateral to eccentric, velutinate, light brown (7.D8), dark brown (8.F7) upon drying, up to 6.0 cm long and 5.0 mm wide; pore surface orange grey (5.B2; 6.B2) to greyish orange (5.B3), pores irregular, round to angular, 1–2/mm, shallow, up to 1 mm deep, decurrent on the stipe and, then, becoming daedaloid, dissepiments thick, entire; margin sterile, context very thin, concolorous with the pore surface, up to 2.5 mm.

Hyphal system dimitic, Generative hyphae simple septate, 2.5–5.0 µm wide, hyaline, in the trama Dm = 3.5, in the context Dm = 3.85, thin to slightly thick-walled, rarely branched, in the stipe Dm = 3.73, pale yellow to brown, with some branches; skeletal hyphae 3.5–7.5 µm wide, unbranched, thick-walled, with wide lumen, dextrinoid (strongly dextrinoid when seen in mass), hyaline to pale cinnamon, in the trama Dm = 4.64, in the context Dm = 5.24

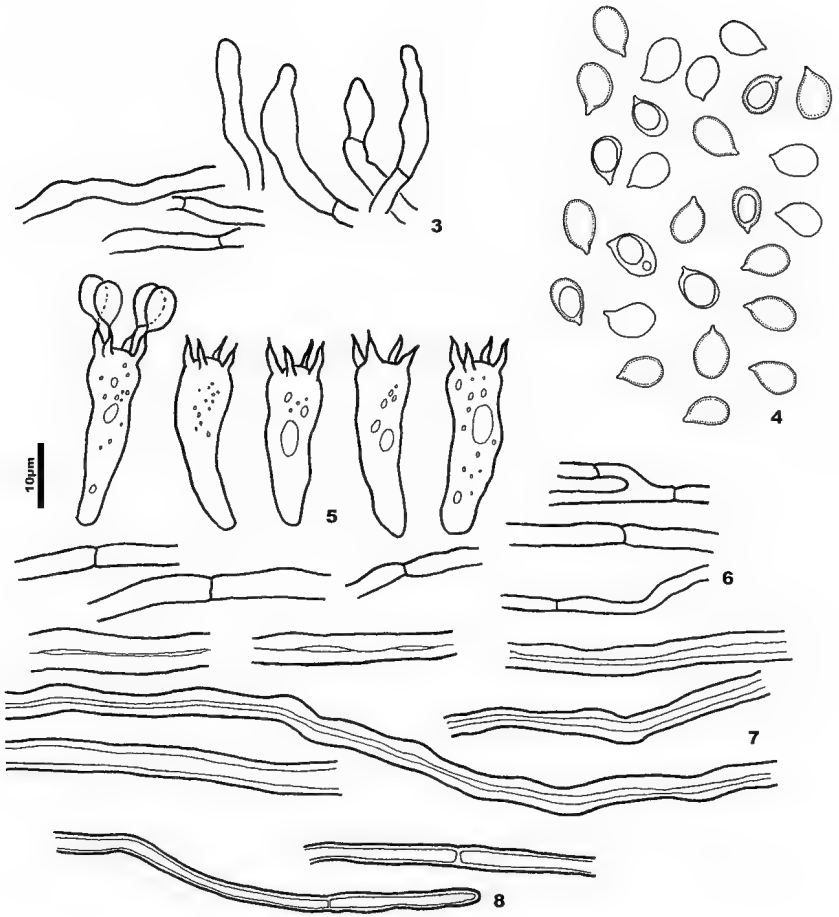
Figs. 1–2. Basidiome of *Microporellus iguazuensis* (ICN 154263)
1. Connected to roots of *Ocotea indecora*, showing the hymenophore. 2. Upper surface.

and parallel, in the stipe Dm = 5.11, pale yellow to rusty brown (all hyphae measurements n = 100/2). Cystidia lacking, but fusoid cystidioles can be found in the dissepiment edges. Basidia clavate 25.0–35.0 × 9.0–10.0 µm, tetraspored, with long sterigmata. Basidiospores broadly ellipsoid to ellipsoid, obovate, asymmetric in side view, smooth and hyaline, thin- to slightly thick-walled, generally with one big oil drop or a sparse oily content, IKI–, acyanophilous, 7.5–9.0 × 5.5–6.5 µm, Lm × Wm = 8.08 × 5.99, Q = 1.23–1.60, Qm = 1.35, n = 100/2.

Culture: Unknown. Attempts to obtain cultures from the context and spore print, using Malt Extract and Potato Dextrose Agars, were unsuccessful.

Substrata: Found on the ground among the litter, but connected to roots of living trees of *Ocotea indecora* (Schott) Mez (*Lauraceae*). Type of rot unknown.

Distribution: Previously known only from the type locality, in the forest of Iguazú National Park, in NE Argentina (Rajchenberg 1987), and now found in the Rio Grande do Sul state, southern Brazil, in two different sites of the Atlantic rainforest. If the species follows the host distribution, it could be widely distributed in forest systems where *O. indecora* (or perhaps other related species of *Ocotea* Aubl.) occur. In Brazil, *O. indecora* is distributed from Bahia to Rio Grande do Sul states (Sobral et al. 2006).



Figs. 3–8. *Microporellus iguazuensis* (ICN 139893).
3. Cystidioles from dissepiment edges. 4. Basidiospores. 5. Basidia.
6. Generative hyphae from trama and context. 7. Skeletal hyphae. 8. Generative hyphae from stipe.

Additional specimen examined: ARGENTINA. Misiones, Parque Nacional Iguazú, ad viam Apepú, 08.III.1980, leg. J.E. Wright M-3379 (BAFC 30708 holotype).

Remarks: *Microporellus iguazuensis* is an interesting polypore distinguished by its lateral to eccentric stipitate basidiome with flabelliform pileus and irregular large pores decurrent on the stipe. Microscopically it is characterized by a dimitic hyphal system with simple septate generative hyphae and strongly dextrinoid skeletal hyphae, large basidia, and ellipsoid to obovate basidiospores. Moreover, it presents a parasitic habit, growing on living roots of laurel trees

(Fig. 1). Macroscopically, *M. iguazuensis* can be confused with some species of *Polyporus* P. Micheli ex Adans. that present a dark stipe and large pores (like *P. guianensis* Mont.). However, *Polyporus* species differ microscopically by clamped generative and non-dextrinoid skeleto-binding hyphae.

Heterobasidion annosum (Fr.) Bref., an important root-rot pathogen of conifers, also presents a dimitic hyphal system with simple septate generative and dextrinoid skeletal hyphae but, in contrast with *M. iguazuensis*, has sessile basidiomes and minutely echinulate basidiospores (Gilbertson & Ryvarden 1986).

Decock (2007) cited the main morphological features of *Microporellus* as follows: light-weight pileate basidiomata possessing a white to pale cream context and trama, a dimitic hyphal system with unbranched skeletal hyphae, non- to dextrinoid vegetative hyphae, and slightly thick-walled spores with cyanophilous walls, generally containing an oil drop. *Microporellus iguazuensis* fits in this circumscription in some characters states, but it is the only species of the genus that combines simple septate generative hyphae, dextrinoid skeletal hyphae and a phytopathogenic habit. Most *Microporellus* species, including the type species, also present ventricose and thick-walled cystidia (Decock 2007), a feature absent in *M. iguazuensis*.

The unique combination of features in *M. iguazuensis* suggests that it should not be included in either *Microporellus* or *Heterobasidion* Bref., but perhaps represents a distinct genus. However, we think that these morphological data are not distinct enough to circumscribe a new genus to accommodate this species; further studies are necessary, including cultural characteristics and molecular data. Our attempts to obtain cultures from the context and spore print, using MEA and PDA, were not successful. Maybe further attempts using other media will succeed in initiating mycelial growth.

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A new species of *Hyphodiscus* (*Helotiales*) on *Stereum*

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ABSTRACT — A new species, *Hyphodiscus stereicola*, is described based on material from Northern Europe, the Canary Islands, and North America. In all of these, greenish apothecia grew on decayed basidiomata of *Stereum* spp. Morphology and host specialisation of the new species are compared with those of other members of the genus.

KEY WORDS — fungicolous ascomycetes, rDNA, taxonomy

Introduction

Hyphodiscus is a genus in the order *Helotiales* characterised by hairy apothecia that are formed on dead, often decorticated, deciduous or coniferous wood. Several species occur on decaying fruitbodies of polypores and corticioid fungi.

Several years ago a light glaucous fungus was found on old basidiomata of *Stereum* in Mexico. The material was sent for identification to the late Ain Raitviir (Tartu, Estonia), who made some hand-written remarks about the delimitation of this fungicolous species as a member of the genus *Hyphodiscus*. Soon a similar specimen was collected in Northern Europe, from Estonia. In autumn 2008, additional material was found from La Gomera (Canary Islands) and Estonia. All four collections are similar in their morphology and host. As these represent a unique combination of characters in the genus *Hyphodiscus*, a new species will be described herein.

Materials & methods

Living ascomata were studied in tap water, 3% aqueous KOH solution, Melzer's reagent (MLZ) and Lugol's solution (LUG). Microphotos and measurements of microscopical elements were taken from living cells of unsquashed freehand sections under cover

glasses. The colour names and codes were adopted from Kornerup & Wanscher (1967). DNA was extracted and the ribosomal DNA ITS regions and 5' end of the large subunit amplified and sequenced according to the protocol described by Põldmaa (2011). The primers ITS1F and LR5 were used for PCR and sequencing. The holotype of the new species is deposited in the mycological herbarium of the Natural History Museum of the University of Tartu (TU). The DNA sequences were deposited to EMBL and the UNITE database (Kõljalg et al. 2005).

Taxonomy

Hyphodiscus stereicola Raitv., Pärtel & K. Põldmaa, **sp. nov.** PLATE 1

MYCOBANK MB 518733

Apothecia gregaria, subsessilia, cupulata, 300 600 µm diametro, margine piloso, viridi-albo, disco olivaceo. Pili cylidracei, obtusi, incrustati, septati, 31–62 × 2–2.8 µm. Excipulum ectale gelatinosum, cellulis venetis; medulla textura intricata composita, brunneolo-aurantiaca. Asci cylindraceo-clavati, octospori, 37–47 × 5–6 µm longi, basi uncinati, poris in solutione iodi non coloratis. Ascosporae hyalinae, ellipsoideae, apicibus obtusis, biguttulatae, 7.6–11.5 × 2.6–4 µm. Paraphyses filiformes, 1.5–2 µm in diametro. Fungicolae ad basidiomata Stereumorum.

HOLOTYPE — **ESTONIA. JÕGEVA CO.**, Puurmani Community, Alam-Pedja Nature Reserve, ca 50 m SW from the beginning of the Kirna hiking trail, mixed forest with dominant *Populus tremula*, 58.544°N, 26.233°E, on decaying basidiomata of *Stereum subtomentosum* Pouzar, 8 Oct 2008, leg. Kadri Põldmaa, TU 104241 [EMBL FN995636].

ETYMOLOGY — named after the host genus.

ECOLOGY — fungicolous, growing on old basidiomata of *Stereum* spp.

APOTHECIA densely gregarious, subsessile to shortly stipitate. Disc almost flat to slightly depressed, 300–600 µm in diameter, olivaceous (1E5). RECEPTACLE at first deeply cup-shaped, later saucer-shaped, externally and at the margin with numerous greenish-white (25A2–3) hairs. Ectal excipulum composed of gelatinous, more or less parallel, slightly interwoven, bluish-green (25B7) pigmented cells terminating above the surface into cylindrical hairs (10–19 × 2–2.2 µm). Medullary excipulum brownish-orange (5C6), of textura intricata. HAIRS greyish green (30C3), mostly straight, cylindrical, slightly pointed, thin-walled, 0–3-septate, 31–62 × 2–2.8 µm, without iodine reaction; wall (except the base) covered with loose irregular-tuberculate greyish green warts. Pigment in excipulum and hair walls dissolving in KOH, apothecia extracting pale yellow hue into KOH solution. ASCI arising from croziers, 8-spored, cylindric-clavate, conically pointed at apex, apical pore not staining in Lugol's or Melzer's reagent (with or without KOH pretreatment), 37–47 × 5–6 µm. ASCOSPORES ellipsoid with obtuse apices, hyaline, smooth, biguttulate (one lipid body at each end, 1–2.8 µm in diameter), aseptate, biseriate or irregularly seriate in asci, 7.6–11.5 × 2.6–4 µm. PARAPHYSES filiform, with 2–3 septa, not exceeding the asci, approximately as long as the asci, 1.5–2 µm in diameter, filled with fine green

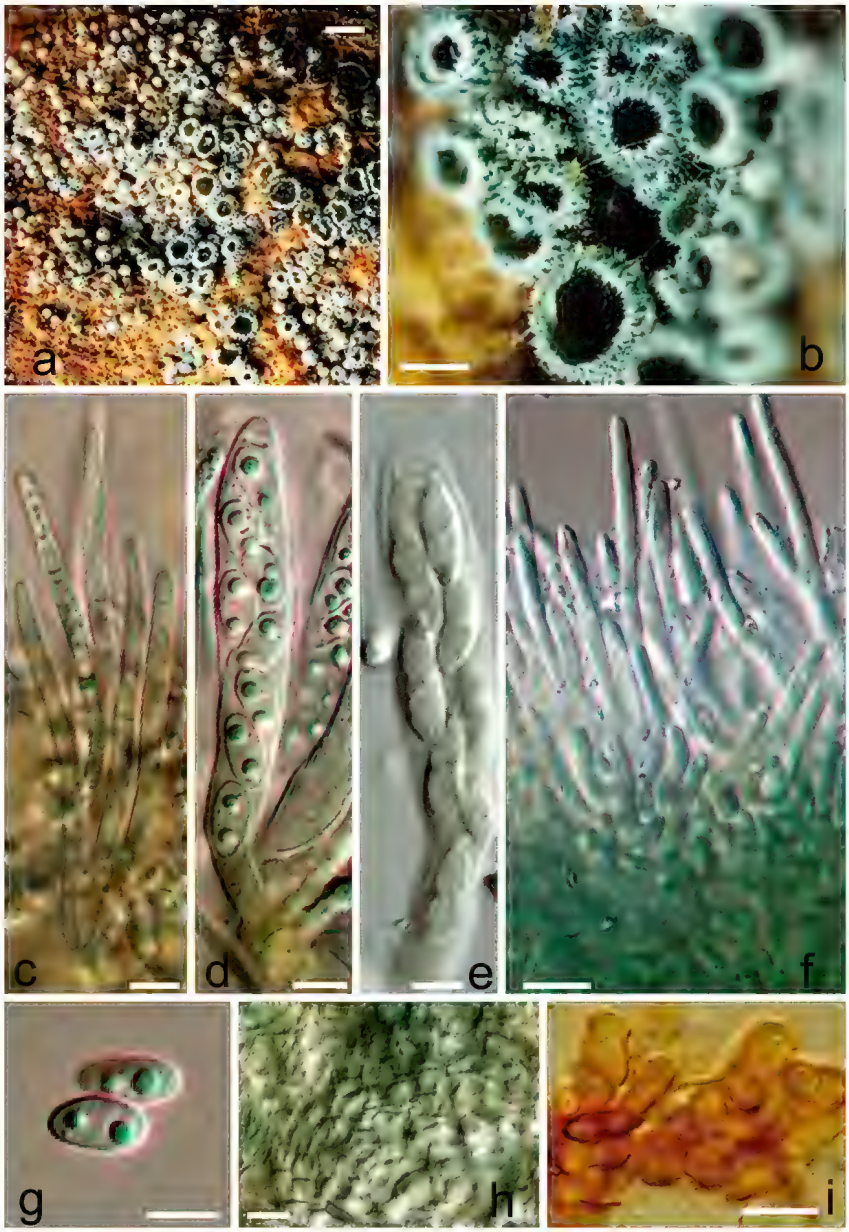

Plate 1. *Hyphodiscus stereicola*. a, b. apothecia; c. paraphyses; d. asci; e. asci (in Melzer's); f. Hairs and upper part of excipulum; g. ascospores; h. ectal excipulum; i. medullary excipulum; (a-d, f, g, i – holotype); e, h – TU 104272. All microscopical subjects (except e) were mounted in water. Scale bars: a – 500 µm; b – 300 µm; c – e, g – 5 µm; f, h, i – 10 µm.

vacuolar bodies, simple or branched only at the base, gradually sligthly tapering towards the apex.

ADDITIONAL SPECIMENS EXAMINED: **ESTONIA. PÄRNU** Co., Saarde Community, W of Reinu, Nigula Nature Reserve, *Picea abies* nemoral forest, 58.04183°N, 24.72383°E, on dead basidiomata of *Stereum subtomentosum*, 26 Aug 2003, leg. I. Parmasto, TAAM 181316. **MEXICO. CHIHUAHUA**, Sierra Madre Occidental, Cascada de Basaseachi, above the left riverbank at the bottom of the waterfall, 28.16944°N, 108.20694°W, alt. ca 1650 m, in mixed forest, on dead basidiomata of *Stereum ostrea* (Blume & T. Nees) Fr., 24 Jul 1994, leg. J. Hafellner 38772, TAAM 200599. **SPAIN. CANARY ISLANDS.** La Gomera, Garajonay National Park, near El Cedro, in laurel forest, 28.13090°N, 17.21319°W, alt ca 800 m, on dead basidiomata of *Stereum ostrea*, 2 Dec 2008, leg. K. Põldmaa; TU 107690 [EMBL FR686966]; leg. K. Pärtel, TU 104272.

COMMENTS. *Hyphodiscus stereicola* clearly differs from other species in the genus due to a unique combination of characters: olivaceous disc, greyish green hairs, and the comparatively large ellipsoidal ascospores. Characteristic of the species is also its fungicolous habit and specialization to old basidiomata of *Stereum*.

It is similar to *H. viridipilosus* (Graddon) Baral which also has green hairs and ellipsoidal ascospores. This species, growing on deciduous wood, is characterized by an ochraceus apothecial disc (Graddon 1974, Baral 1993). The asci and ascospores are smaller (ca 3–4 × 1.5–2 µm) than in *H. stereicola*. There are several yet undescribed species shown in Hans Otto Baral's detailed drawings (Baral & Marson 2005), none of which seems to resemble *H. stereicola*.

Hyphodiscus stereicola reveals substrate specificity, as all the specimens have been collected on *Stereum* spp. Found either on the pileus or on the hymenium, it appears to prefer decaying basidiomata. In the two collections from boreal forests in Estonia the host is *S. subtomentosum*. The morphologically very similar species, *S. ostrea* having a southern distribution, serves as the host for the other specimens, collected in Mexico and the Canary Islands. In Estonia and La Gomera the collection habitats represent primeval forests with many fallen rotting trunks.

Although the fungicolous lifestyle is rare among the *Helotiales*, members of *Hyphodiscus* tend to grow on fruitbodies of basidiomycetes. The most common of these, *H. hymeniophilus* (P. Karst.) Baral, grows on decaying basidiomata of *Antrodia serialis* (Fr.) Donk, *Antrodiella hoehnelii* (Bres.) Niemelä, *Piptoporus betulinus* (Bull.) P. Karst., *Polyporus varius* (Pers.) Fr. and *Trametes versicolor* (L.) Lloyd (Helfer 1991). *Clavidisculum stereicola* (Cooke) Raitv., later synonymised with *H. hymeniophilus* by Raitviir (2004), was reported to grow on *Stereum* sp. (Raitviir 1970).

In addition, *H. theiodeus* (Cooke & Ellis) W.Y. Zhuang occurs on *Peniophora* spp. (Zhuang 1988) and other wood-decaying basidiomycetes, while *H. incrustatus* (Ellis) Raitv. has been found on old *Polyporus* spp. (Raitviir

2004). Hosoya et al. (2010) speculated that fungicolous association might stimulate formation of ascomata in the whole genus, as suggested by the frequent observation of other fungi near *Hyphodiscus* fruitbodies.

Hyphodiscus stereicola is one of the few species of apothecial ascomycetes that is found on basidiomata of species of *Stereum*. At the same time members of this basidiomycete genus serve as hosts to a number of representatives of the *Hypocreales*, the ascomycete order richest in fungicolous associations. Besides several species of *Hypomyces* (Põldmaa 2003, Rogerson & Samuels 1993) and *Sphaerostilbella*, these include also *Nectriopsis oropensoides* (Rehm) Samuels. Despite the overall low host-specificity of members of these fungicolous genera growing on aphyllophoroid basidiomycetes (Põldmaa 2000, Rogerson & Samuels 1993), the majority of the species inhabiting *Stereum* are found only on basidiomata of this genus. The annual, dimitic, comparatively soft basidiomata apparently provide a specific niche, in contrast to the mostly perennial, di- or trimitic, tough basidiomata of many aphyllophoroid genera which host other fungicolous ascomycetes, including *Hyphodiscus*. Such specialisation could also be explained by the phylogenetic component as the genus *Stereum* (*Russulales*) is not closely related to most of the other aphyllophoroid genera distributed across the *Agaricomycetes*.

The asexual stages of *Hyphodiscus* species have been described in the anamorph genus *Catenulifera*: *C. rhodogena* (F. Mangenot) Hosoya for *H. hymeniophilus* and unnamed *Catenulifera* spp. for *H. pinastri* R. Galán & Raitv., *H. theiodeus*, and *H. otanii* Hosoya (Galán & Raitviir 2004, Hosoya 2002). Our attempts to isolate the most recent collections into pure culture were not successful.

The genus *Hyphodiscus* has been accepted as a member of *Hyaloscyphaceae* by most authors (Verkley 1993, 1996; Hosoya 2002; Raitviir 2004, Hosoya et al. 2010) with unclear affinities based on rDNA sequences (Untereiner et al. 2006, Bogale et al. 2010). However, rDNA ITS and SSU sequences reveal that *H. hymeniophilus* is related to members of *Hyaloscyphaceae* and *Dermateaceae* but also to anamorphic helotialean root endophytes like *Phialocephala (Vibrisseaceae)* (Untereiner et al. 2006, Bogale et al. 2010). The true phylogenetic relationships of *Hyphodiscus* species are currently being clarified (R. Galán pers. comm.). The rDNA ITS region of the two Estonian specimens of *H. stereicola* were identical, whereas that of the La Gomeran material differed in 6 bp. A BLAST search of ITS regions found only weak correspondence between the type of *H. stereicola* and databased sequences from identified species (a best match of 84% with *H. hymeniophilus* and *Catenulifera brachyconia* (W. Gams) Bogale & Unter.). However, the true phylogenetic affinities of *H. stereicola* remain unclear due to the lack of sequence data for most of the species of this and related genera.

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Chaetomium siamense sp. nov., a soil isolate from Thailand, produces a new chaetoviridin, G

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Abstract — *Chaetomium siamense* sp. nov., isolated from soil in a pineapple plantation in Thailand, is described and illustrated. The new species is similar to *C. cupreum* in having superficial globose or ovate ascomata with red circinate ascomatal hairs but can be distinguished by its fusiform ascospores with two apical germ pores. Phylogenetic analysis of ITS rDNA sequence data also supports *C. siamense* as distinct. Investigation of the secondary metabolites of *C. siamense* led to the isolation of one new (chaetoviridin G) and seven known compounds (ergosterol, 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol, ergosterylplamitate, cochliodone D, chaetoviridin A, chaetoviridin F, chrysophanol).

Key words — *Ascomycota, Chaetomiaceae,* novel compound

Introduction

Chaetomium Kunze, established in 1817, is an ascomycete genus in the family *Chaetomiaceae* (von Arx et al. 1986). *Chaetomium* is one of the largest genera of saprotrophic ascomycetes, comprising more than 300 species worldwide (Seth 1970, von Arx et al. 1986, Cano & Guarro 1987, Carris & Glawe 1987, Moustafa & Ess El-Din 1989, Horie & Udagawa 1990, Abdullah & Zora 1993, Udagawa et

al. 1994, Gené & Guarro 1996, Decock & Hennebert 1997, Udagawa et al. 1997, Rodríguez et al. 2002, Wang & Zheng 2005a,b). *Chaetomium* is characterized by superficial ascomata, usually covered with hairs or setae, surrounding a rather wide ostiolar pore at the apex. The ascospores are aseptate, brown or gray-olivaceous, and with one or two germ pores (von Arx et al. 1986).

During a study on antagonistic fungi for controlling root rot disease of pineapple, numerous strains of *Chaetomium* were isolated from soil using a baiting technique (Soytong & Quimio 1989). Although several species have been only recently recorded for Thailand (Pornsuriya et al. 2008), one isolate could not be identified as any known *Chaetomium* species. This isolate is here described and illustrated as *Chaetomium siamense* sp. nov., and is compared with *C. cupreum*, the most similar species. Molecular evidence, also provided, supports this species as an independent taxon.

Materials & methods

Sampling, isolation and morphological study

Soil samples were collected from pineapple plantations in Phatthalung Province, Thailand, during August to November 2007. They were placed in clean plastic bags for transport to the laboratory at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. *Chaetomium* species were originally isolated by the baiting technique described by Soytong & Quimio (1989). *Chaetomium* species developing on baits were observed daily and their ascomata transferred to glass slides with a small amount of sterilized water before being spread on water agar (WA) in a 9-cm diameter Petri dish and then incubated for 24 h at room temperature, Individual colonies were transferred onto potato dextrose agar (PDA) and corn meal agar (CMA) for identification. Morphological structures of the new species were observed under compound and scanning electron microscopes. The holotype is deposited in Biocontrol Research Unit and Mycology Section (KMITL).

DNA extraction, polymerase chain reaction (PCR) and data analysis

Genomic DNA was extracted from a mycelial culture grown in potato dextrose broth (PDB) by using Dneasy Plant Mini kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS1, 5.8S and ITS2 regions) was performed with primer pair PN3 and PN16 (Neuvéglise et al. 1994). The reaction was carried out in 25 µl volumes containing 1 µl genomic DNA, 0.5 µl dNTPs, 1 µl of each primer and 0.2 µl Taq DNA polymerase in 2.5 µl PCR buffer. Amplifications were performed with an initial denaturation step of 95°C for 5 min followed by 35 cycles of 94°C for 1 min, 60°C for 2 min and 72°C for 3 min and a final extension of 72°C for 5 min. The PCR product was separated on an agarose gel and purified for DNA sequencing by the U-gene Gel Extraction Kit II (U-gene Biotechnology Co, P.R China). DNA sequences of related species, based on the ITS including 5.8S gene data, were retrieved from GenBank (Table 1). *Chaetomium nigricolor* and *Chaetomium* sp. 15004 were used as outgroup. All sequences were assembled using BioEdit ver. 7.0.2 and aligned using ClustalX ver 1.83. Phylogenetic relationships were determined by

TABLE 1. *Chaetomium* species and isolates used in this study

ISOLATES	ITS GENBANK ACCESSION NO.	HOST/SUBSTRATE	GEOGRAPHIC ORIGIN
C. atrobrunneum	FJ595483	Chinese child	China
C. bostrychodes	EU520130		China
C. cupreum	AB509366	soil	Thailand
	AB509367	soil	Thailand
	AB509368	soil	Thailand
	AB509369	soil	Thailand
	AB509370	soil	Thailand
	AB509371	soil	Thailand
	AB509373	soil	Thailand
C. funicola	EU552109	*Leucadendron* sp.	South Africa
	EU139246	*Macrotermitinae* nest	France
C. globosum	FJ538594	chrysanthemum	USA
C. hispanicum	DQ069021	*Picea abies*	Lithuania
C. longicolleum	GQ922525		Netherlands
C. murorum	GQ376100	deteriorated painting	Greece
C. nigricolor	EU543258	*Dioscorea zingiberensis*	China
C. pachypodioides	GQ922526		Netherlands
Chaetomium sp. 15004	EU750692		China
Chaetomium sp. aurim1182	DQ093660	*Picea abies*	Lithuania
Chaetomium sp. INBI2 26( )	AJ620951	soil	Vietnam
C. thermophilum	EF550983		India

heuristic search under neighbor joining (NJ) using PAUP* 4.0b8 (Swofford 2001). Tree was drawn using Treeview.

Identification of metabolites

Erlenmeyer flasks (1 L) containing 200 ml PDB were inoculated with mycelial plugs from a 5-day-old culture of *C. siamense*. The flasks were incubated for 4 weeks on stationary state at 27–30°C. Mycelium mats were then harvested, air dried, ground, and extracted with a series of methanol, ethyl acetate and hexane. The crude extract was loaded onto a silica gel column. The column was successively eluted to obtain different fractions and monitored by TLC, and reduced to one active fraction. The chemical structures isolated from dried mycelial mats of *C. siamense* were elucidated on the basis of spectroscopic evidence (IR, ^{1}H NMR, and ^{13}C NMR and 2D-NMR).

Taxonomy & discussion

Chaetomium siamense Pornsuriya & Soytong, **sp. nov.** FIGS 1

MYCOBANK MB 506801

Coloniae modica bardus crescents in agaris PDA et CMA, cum mycelio aerio; reversum rubus. Mycelium ex hyphis hyalinis vel subhyalinis, septatis, laevibus, 1.8 2.3 µm diam compositum. Ascomata superficialia, globosae, ovata, ostiolata, fuscobrunneum in refulgens, paramariae ad 87 148 × 107 177 µm. Peridium fuscobrunneum et ex textura angularis; epidermoidea ex fuscus, paramariae ad 2.8 5.7 × 5.1 10.4 µm. Ascomatal pili abundantibus, simplici, aurantiacus in refulgens, spira in ultima parte et ex septati,

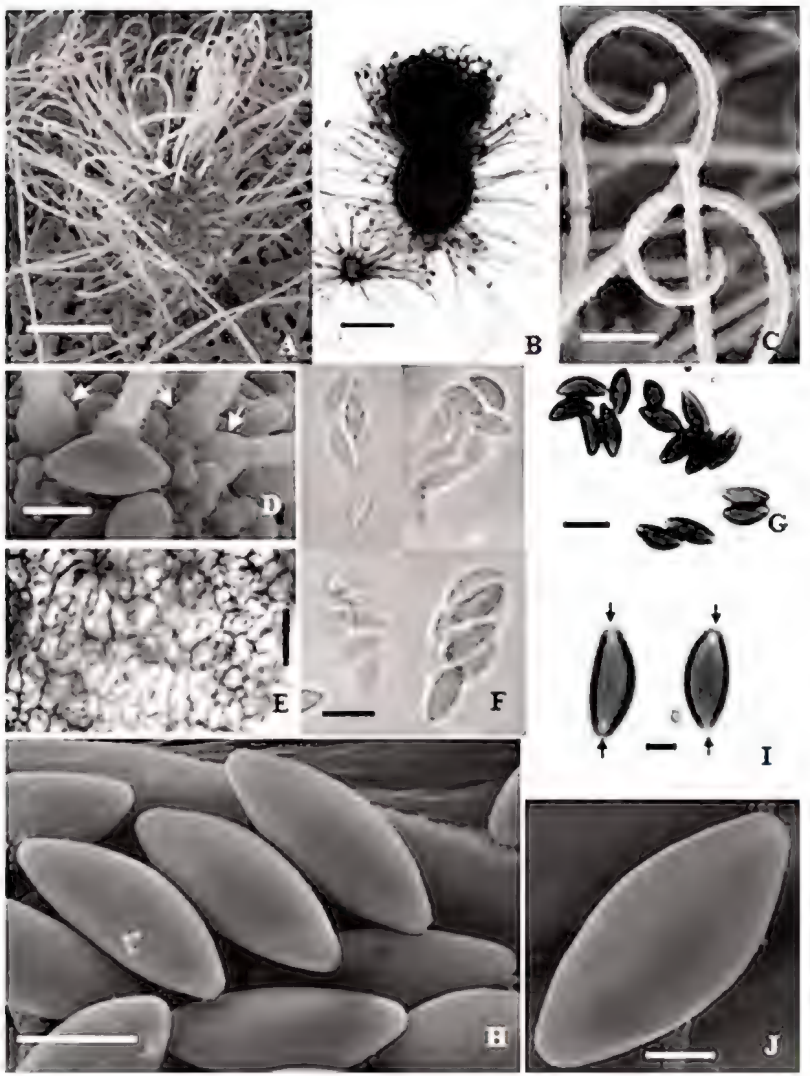

FIGURE 1. *Chaetomium siamense*. A. Ascoma viewed under a scanning electron microscope. B. Ascoma viewed under a light microscope. C–D. Arcuate ascomatal hairs with swollen basal cells (arrowed) viewed under a scanning electron microscope. E. Peridium viewed under a light microscope. F. Asci viewed under a light microscope. G. Ascospores viewed under a light microscope. H. Ascospores viewed under a scanning electron microscope. I. Fusiform ascospores with two apical germ pores (arrowed). J. Ascospore viewed under a scanning electron microscope. Bars: A, B = 50 µm; C, E–G = 10 µm; D, H = 5 µm; I, J = 2 µm.

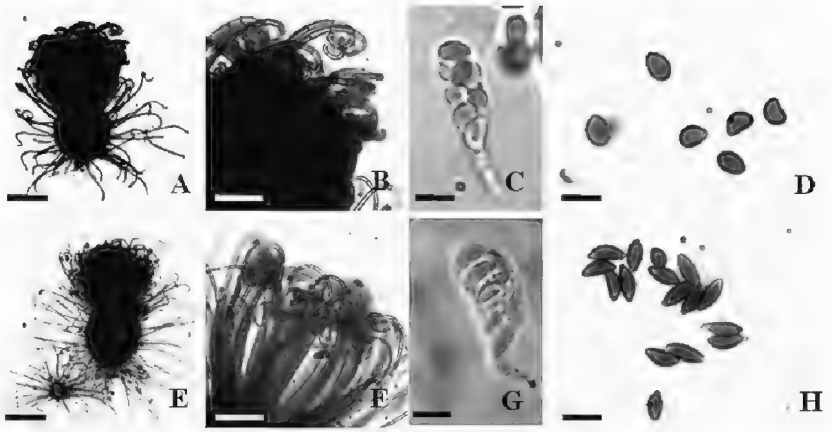

FIGURE 2. Comparison of the characteristics of *Chaetomium cupreum* (A–D) and *C. siamense* (E–H). Bars: A, E = 50 µm; B–D, F–H = 10 µm.

1.3–4 µm diam, 170 µm longi. Pili laterals recti, leviter, verrucosi, paramariae ad 56–160 µm longi. Asci numerosis, fasciculati, clavati, pedicellati, 8-spori, 9–14 × 28–40 µm. Ascosporae fusiformia, fascobrunnneum, laevibus, 4.3–5 × 11.1–13.5 µm, cum 2 poro germinalibus apicali visibile.

HOLOTYPE: Thailand, Phatthalung, isolated from soil in a pineapple plantation, 27 September 2007, Chaninun Pornsuriya TMACC001, deposited in herbarium of the Biocontrol Research Unit and Mycology Section, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok 10520, Thailand.

ETYMOLOGY: *siamense* refers to Thailand (Siam), country of origin.

Colonies on PDA and CMA growing moderately slowly, covering 9 cm Petri dish surface after 14 days at room temperature, with aerial mycelium; becoming red due to red pigment exudates when young, yellow when old and formation of ascomata. Mycelium composed of hyaline to subhyaline, septate, smooth hyphae, 1.8–2.3 µm diam. Ascomata discrete, maturing within 10–14 days, superficial, globose or ovate, ostiolate, dark brown in reflected light, 87–148 × 107–177 µm. Peridium dark brown, of textura angularis; outer layer composed of brown, thick-walled cells measuring 2.8–5.7 × 5.1–10.4 µm. Ascomatal hairs abundant, unbranched, red or orange-red in reflected light, apically circinate or coiled, regularly septate and forming a dense tuft around the ostiole, finely verrucose towards the tip, up to 170 µm long, 1.3–4 µm diam near the base. Lateral hairs less abundant, straight, finely verrucose, 56–160 µm long. Asci numerous, fasciculate, clavate, stalked, 8-spored, 9–14 × 28–40 µm. Ascospores fusiform, dark brown at maturity smooth and rather thick-walled, 4.3–5 × 11.1–13.5 µm, with two apical germ pores.

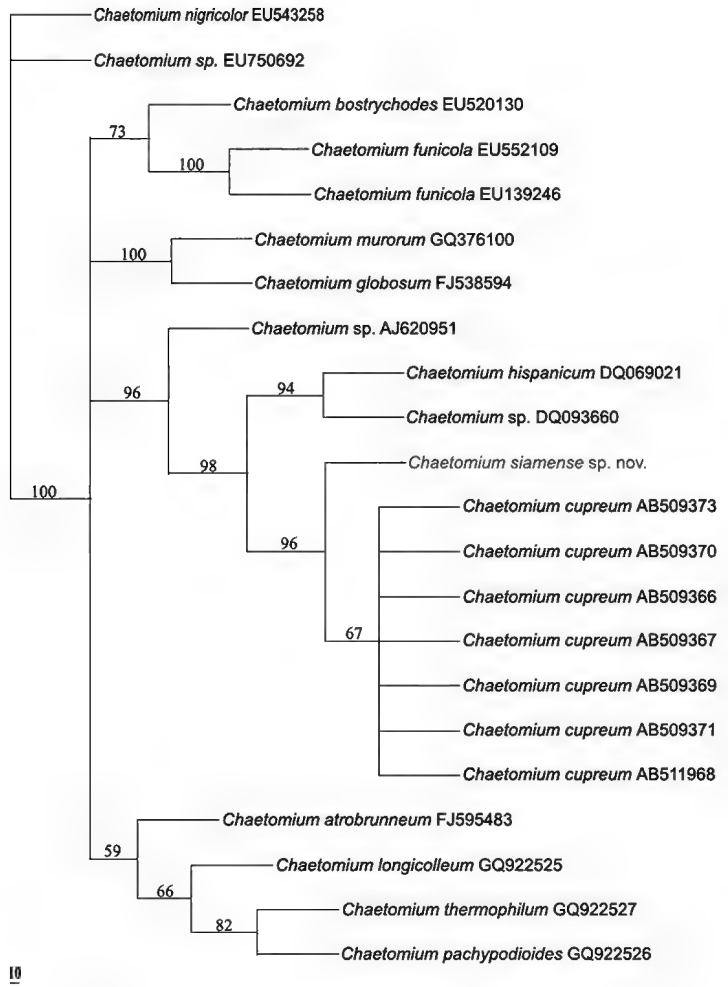

FIGURE 3. Phylogenetic tree of *Chaetomium* spp. from GenBank including *Chaetomium siamense* constructed after distance-based analysis of ITS1 and 5.8S regions of rDNA. Numbers at the branches indicate the percentage of bootstrap values after 1000 replications. The outgroup taxa are *Chaetomium nigricolor* and *Chaetomium* sp.

COMMENTS: *Chaetomium* is a large ascomycete genus with species inhabiting various cellulose-based substrates including paper and plant compost. After review of the genus by von Arx et al. (1986) several new species have been described: *C. biporatum* (Cano & Guarro 1987), *C. histoplasmoides* (Carris & Glawe 1987), *C. sinaiense* (Moustafa & Ess El-Din 1989), *C. subcurvisporum*

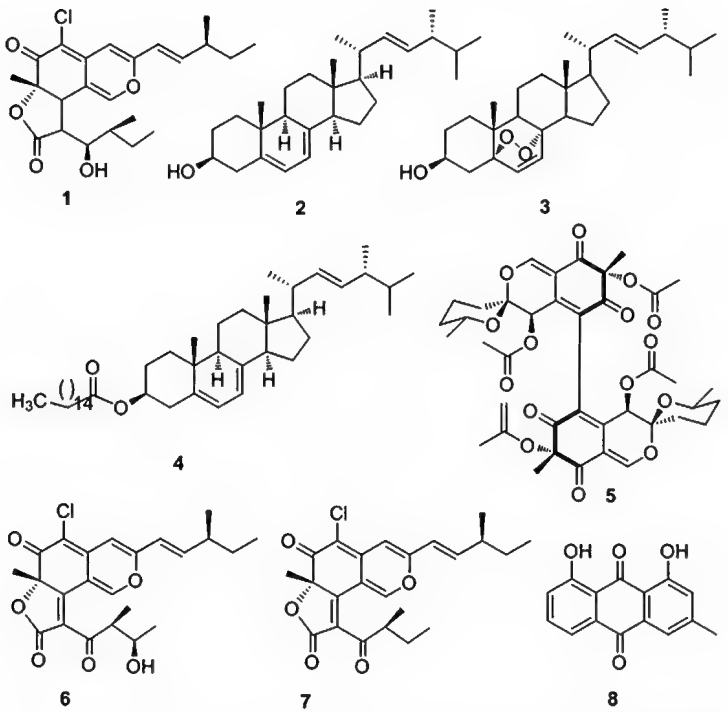

FIGURE 4. Compounds isolated from *Chaetomium siamense*. 1. chaetoviridin G; 2. ergosterol; 3. 24(R)-5α,8α-epidioxyergosta-6-22-diene-3β-ol; 4. ergosterylplamitate; 5. cochliodone D; 6. chaetoviridin A; 7. chaetoviridin F; 8. chrysophanol.

(Abdullah & Al-Bader 1989), *C. myricicola* (Horie & Udagawa 1990), *C. mesopotamicum* (Abdullah & Zora 1993), *C. novae-caledoniae* (Udagawa et al. 1994), *C. floriforme* (Gené & Guarro 1996), *C. cuyabenoensis* (Decock & Hennebert 1997), *C. umbratile* (Udagawa et al. 1997), *C. macrostiolatum*, *C. olivicolor*, *C. tarraconense* (Rodríguez et al. 2002) *C. acropullum* (Wang & Zheng 2005a), and *C. ampulliellum* (Wang & Zheng 2005b). *Chaetomium siamense* can be distinguished from other species by its superficial, spherical or ovate ascomata with angular brown walls. Ascomatal hairs are red or orange-red in reflected light, arcuate, apically circinate or coiled, septate and ascospores are fusiform with two apical germ pores. The new species was initially identified as *C. cupreum* L.M. Ames, which produces red pigment in agar medium and which also produces superficial spherical or ovate ascomata (FIGS. 2A,E) with a peridium of angular brown-walled cells.

Ascomatal hairs are red, arcuate, apically circinate or coiled and septate (FIGS. 2B,F). However, ascospore morphology easily separates the new species

from *C. cupreum* (Figs. 2H,D). The longer (11.1–13.5 µm) fusiform ascospores with two apical germ pores (Fig. 2H) of *C. siamense* clearly differ from the shorter (7–10 µm) reniform ascospores with a single apical germ pore (Fig. 2D) that characterize *C. cupreum*. Although the phylogenetic analysis indicates that *C. siamense* is related to *C. cupreum*, sequence differences (as shown by the separate branch tips, Fig. 3) clearly support *C. siamense* as a distinct species.

Investigation of the secondary metabolites from crude extracts of dried mycelial mats of *C. siamense* led to the isolation of a new compound named chaetoviridin G (1). Seven other known compounds — ergosterol (2), 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol (3), ergosterylplamitate (4), cochliodone D (5), chaetoviridin A (6), chaetoviridin F (7), and chrysophanol (8) — were also identified. Structures were elucidated based on spectroscopic evidence (IR, ^{1}H NMR, and ^{13}C NMR and 2D-NMR). Compounds 2–8 (Fig. 4) have also been reported from *C. cochliodes* (Phonkerd et al. 2008).

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New taxa and new reports of *Phyllopsora* (lichenized *Ascomycotina*) from India

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ABSTRACT An account of 14 species of *Phyllopsora* from India is provided. *P. catervisorediata* and *P. himalayensis* are described as new species, while *P. corallina* var. *subglaucella* as a new variety. *P. albicans, P. breviuscula, P. chlorophaea, P. confusa, P. isidiotyla, P. kalbii, P. mauritiana, P. nemoralis, P. subcrustacea,* and *P. swinscowii* are new records for the Indian lichen biota.

KEY WORDS *Ramalinaceae, Ascomycetes,* squamulose, taxonomy

Introduction

The genus *Phyllopsora* is characterised by a crustose to squamulose thallus, thin to thick, white to brown to reddish brown prothallus, minute to subfoliose squamules which are corticated on upper side, green alga photobiont, convex, biatorine apothecia with a hyaline to pale yellow epihymenium, hyaline to reddish-brown exciple, hyaline to yellowish-brown hymenium, hyaline or golden yellow to brownish hypothecium, simple to conglutinated paraphyses, 8-spored asci and hyaline, simple, ellipsoid, bacilliform to fusiform ascospore. The species of *Phyllopsora* mostly inhabit bark but sometimes grow on rock and bryophytes. The species exhibit wide ranges of altitudinal distribution from sea level up to an height of 3000 m.

Phyllopsora (*Ramalinaceae*) is a common lichen genus in tropical and subtropical areas of the world. Swinscow & Krog (1981) revised the East African species of *Phyllopsora* and reported the occurrence of eleven species from the region. Brako (1989, 1991) monographed the neotropical flora and enumerated eighteen species and eight varieties. Timdal & Krog (2001) studied

the East African and Mascarene *Phyllopsora* species. Elix (2006a, b) recorded five new species of *Phyllopsora* from Australia and for the first time Timdal (2008) described sorediate *Phyllopsora* species from Peru.

Upreti et al. (2003) described five species of *Phyllopsora* from India. Later, Awasthi (2007) updated the occurrence of six taxa of *Phyllopsora* from India, where *P. kiiensis* (Vain.) Gotth. Schneid. was considered to be a synonym of *P. haemophaea* (Nyl.) Müll. Arg. However, it is clear from our study and according to Timdal & Krog (2001) that *P. kiiensis* is a distinct species, while *P. haemophaea* is a synonym of *P. furfuracea* (Brako 1991). The known taxa of *Phyllopsora* from India prior to the present communication include *P. buettneri* (Müll. Arg.) Zahlbr., *P. corallina* (Eschw.) Müll. Arg., *P. furfuracea, P. kiiensis, P. manipurensis* (Müll. Arg.) Gotth. Schneid., *P. parvifolia* (Pers.) Müll. Arg. var. *parvifolia*, and *P. parvifolia* var. *subgranulosa* (Tuck.) Müll. Arg. The present investigation adds further 13 taxa of *Phyllopsora* to the Indian lichen biota, including two new species and one new variety. The description and illustration of *P. furfuracea* is also provided here for better understanding of the taxa.

Materials & methods

The present study is based on the lichen specimen preserved at the herbarium of the National Botanical Research Institute, Lucknow (LWG), which also includes the personal herbarium of Dr. Awasthi (LWG-AWAS). The specimens were examined morphologically, anatomically, and chemically. Thin hand-cut sections of apothecia and thallus were mounted in plain water, cotton blue and 5% KOH and observed under a compound microscope. For chemical spot tests the usual reagents of K, C and P were used. TLC was performed in solvent system A, following Walker & James (1980). Swinscow & Krog (1981) was followed for the terminology of cortex types.

Key to the Indian *Phyllopsora* taxa

1. Thallus isidiate or sorediate 2
1a. Thallus otherwise 11
2. Thallus sorediate ..... *P. catervisorediata*
2a. Thallus isidiate 3
3. Isidia mostly globular 4
3a. Isidia mostly cylindrical 5
4. Thallus greenish, upper cortex type 2; apothecia brown to black, K–.... *P. kalbii*
4a. Thallus yellowish, upper cortex type 1–2; apothecia dark brown, K + yellow ..... *P. himalayensis*
5. Squamules 0.3–1.5 mm wide 6
5a. Squamules 0.1–0.3 mm wide 8
6. Thallus containing methyl 2,7-dichloropsoromate and methyl 2,7-dichloronorpsoromate ..... *P. swinscowii*

6a. Thallus chemistry otherwise 7
7. Thallus containing furfuraceic acid ..... *P. kiiensis*
7a. Thallus lacking furfuraceic acid ..... *P. corallina* var. *corallina*
8. Thallus K–, containing furfuraceic acid ..... *P. furfuracea*
8a. Thallus K+ yellow, lacking furfuraceic acid 9
9. Isidia mostly bulbate at the base, thick; apothecia brown ..... *P. corallina* var. *subglaucella*
9a. Isidia simple or coralloid, common to abundant; apothecia brown to black ... 10
10. Isidia thick, irregular shaped; upper cortex thin, gelatinous ..... *P. isidiotyla*
10a. Isidia thin, short; upper cortex type 1–2 ..... *P. nemoralis*
11. Squamules 0.1–0.3 mm wide 12
11a. Squamules 0.3–1.5 mm wide 16
12. Prothallus reddish brown to black; squamules ascending ..... *P. chlorophaea*
12a. Prothallus white or red; squamules adnate to ascending 13
13. Prothallus red; hypothecium colourless ..... *P. parvifolia* var. *subgranulosa*
13a. Prothallus white; hypothecium yellowish to reddish brown 14
14. Squamules yellowish to greenish; hypothecium dark brown ..... *P. manipurensis*
14a. Squamules greenish; hypothecium golden yellow to reddish brown 15
15. Ascospores 11–18 × 2–3 µm; apothecia with fibrillose margin ... *P. subcrustacea*
15a. Ascospores 8–11 × 1–2 µm; apothecia glabrous margin ..... *P. confusa*
16. Thallus PD+ orange, red 17
16a. Thallus PD– 18
17. Squamules mostly pruinose, strongly pubescent, not imbricate ..... *P. buettneri*
17a. Squamules not pruinose, rarely pubescent, imbricate ..... *P. albicans*
18. Squamules mostly ascending, upper side yellowish brown, pubescent at margin ..... *P. parvifolia* var. *parvifolia*
18a. Squamules adnate to ascending, upper side greenish, margin occasionally pubescent 19
19. Squamules closely adnate, overlapping; hypothecium colourless to pale brown ..... *P. mauritiana*
19a. Squamules ascending; hypothecium golden yellow to brown ..... *P. buettneri*

The species

Phyllopsora albicans Müll. Arg., Bull. Soc. R. Bot. Belg. 32: 132 (1893). FIG. 1

Prothallus thin to thick, reddish brown. Thallus squamulose, ascending, mainly imbricate, elongate, incised to deeply divided, 0.3–1.0 mm wide; upper surface glabrous to slightly pubescent, pale green to dark green, plane to convex, margin pubescent. Isidia absent. Cortex 1–2 type, 12–20 µm thick; medulla containing crystals, dissolving in K.

Apothecia rare, up to 1.5 mm diam.; disc plane to convex, reddish brown; margin raised. Exciple reddish-brown; epihymenium hyaline; hymenium colourless to pale yellow; hypothecium golden yellow and containing crystals which dissolve in K. Ascospores hyaline, simple to narrowly ellipsoidal to bacilliform, 6–10 × 1–2 µm. Pycnidia not seen.

CHEMISTRY — Thallus K–, C–, KC–, PD+ yellow orange; argopsin and pannarin in TLC.

SPECIMENS EXAMINED — **INDIA: Arunachal Pradesh**, UPPER SIANG DISTRICT, Jengging, Apanavan area, alt. 1014 m, on bark, 18.11.2008, D.K. Upreti, U. Dubey, R. Khare & G. Mishra 08-009322 (LWG), WEST SIANG DISTRICT, Eyi village, on bark, 17.10.2007, U. Dubey s.n. (LWG), **Kerala**, PALGHAT DISTRICT, Silent Valley National Park, on *Mesua ferea* bark, 24.11.2006, B. Haridas 06-014751 (LWG).

REMARKS — *Phyllopsora albicans* is distinguished by the elongated, often imbricate squamules. It is close to *P. buettneri* in having a PD+ orange reaction (pannarin or related compounds), but the later species differs in having pruinose, strongly pubescent squamules containing additionally zeorin. *P. albicans* was earlier reported from the montane habitats of Africa, Costa Rica, Philippines and Taiwan, between altitudes of 540–2000 m (Swinscow & Krog 1981, Timdal & Krog 2001). In India, the species is distributed in tropical to subtemperate areas in the state of Arunachal Pradesh and Kerala between altitudes 500–1500 m. It is a new record for the country.

Phyllopsora breviuscula (Nyl.) Müll. Arg., Bull. Herb. Boissier 2(App. 1): 45 (1894). FIG. 2

≡ *Lecidea breviuscula* Nyl., Annls Sci. nat., Bot., ser. 4, 19: 339 (1863).

Prothallus thick and reddish brown. Thallus squamulose; squamules adnate, ascending to elongate, crenulate to incised 0.3–1.0 mm wide; upper surface glabrous, pale green to green, plane to convex, sometime fibrillose at the margin. Isidia absent. Cortex 2 type, 20–35 µm thick; medulla containing crystals dissolving in K.

Apothecia common, up to 1.5 mm in diam.; disc plane to convex, reddish-brown; margin slightly raised and glabrous; exciple pale brown; epihymenium hyaline to pale yellow; hymenium hyaline; hypothecium reddish brown, containing crystals which dissolve in K. Ascospores hyaline, simple to fusiform, 6–11 × 2–3.5 µm. Pycnidia not seen.

CHEMISTRY — Thallus K–, C–, KC–, PD–; no lichen substance in TLC.

SPECIMENS EXAMINED — **INDIA: Karnataka**, CHIKMAGALURE DISTRICT, Chamudi Ghat, Kuvettu, alt. 104 m, on bark of trees in evergreen forest, 15.01.2008, H.T. Lumbsch, D.K. Upreti & P.K. Divakar 19740/B (LWG).

REMARKS — *Phyllopsora breviuscula* is distinguished by the large, partly imbricate squamules and the reddish brown hypothecium. The Indian specimen

contains crystals in medulla and hypothecium, but no lichen substances were detected in TLC. It is close to *P. parvifolia* in having large squamules and reddish brown prothallus but the latter species differs in forming more plane squamules with phyllidia on the lobe margins in the inner part of the thallus. *P. breviuscula* was earlier known from Cuba, Mauritius, and La Réunion between altitudes of 300–1390 m (Timdal & Krog 2001). In India, the species is collected from tropical areas of Karnataka in western Ghat between altitudes of 100–500 m, and is a new species from the country.

Phyllopsora catervisorediata G.K. Mishra, Upreti & Nayaka, sp. nov. FIG. 3

MYCOBANK MB 518498

Thallus squamulosus; squamulae ascendentes, elongatae, luteo-virescentes, sorediatae. Apothecia ignota.

HOLOTYPE INDIA: Uttarakhand, BAGESHWAR DISTRICT, enroute to Pindari glacier, from Dwali to Khati, alt. 2734 3210 m, on bark, 13.05.2007, S. Joshi & Y. Joshi 07-008932 (LWG).

ETYMOLOGY: From the latin noun *caterva* (group or heap), referring to the appearance of the soredia.

Prothallus indistinct. Thallus squamulose, closely adnate, rounded to elongate, sometimes ascending, 0.1–0.5 mm wide; upper surface glabrous, pale green to yellowish, plane to convex, epruinose, sorediate. Soredia farinose, developing from the margin of squamules, forming heaps. Cortex of type 2, 5–10 µm thick; medulla containing crystals dissolving in K.

Apothecia and pycnidia not seen.

CHEMISTRY — Thallus K+ yellow, C–, KC–, PD–; atranorin in TLC.

REMARKS — *Phyllopsora catervisorediata* is characterized by the marginally sorediate squamules and the indistinct prothallus. The soredia forms heaps on the margin of the squamules. It is close to *P. soralifera* Timdal, in having sorediate squamules and cortex of type 2, but the latter species differs in having capitate soredia and in lacking lichen substances. *P. catervisorediata* is known from its type locality in the Western Himalayas, where it was found growing on bark of tree in moist and humid forest at an altitude of 2730 m.

Phyllopsora chlorophaea (Müll. Arg.) Zahlbr., Denkschr. Akad. Wiss. Wien 83: 133 (1909). FIG. 4

≡ *Psora chlorophaea* Müll. Arg., Flora 70: 320 (1887).

Prothallus reddish brown to black. Thallus squamulose, ascending, overlapping, elongate, minute 0.1–0.3 mm wide; upper surface glabrous above, pale brown, plane to convex, sometime fibrillose at the margin. Isidia absent. Cortex of type 1–2, 5–10 µm thick; medulla containing crystals that dissolve in K.

Apothecia and pycnidia not seen.

CHEMISTRY — Thallus K–, C–, KC–, PD–; ± atranorin in TLC.

SPECIMENS EXAMINED — **INDIA: Karnataka**, SHIMOGA DISTRICT, Sharavati Ghat, near Gersoppa, alt. 718 m, in evergreen forest, on bark, 16.01.2008, H.T. Lumbsch, D.K. Upreti & P.K. Divakar 19744Q (LWG), **Orissa**, GANJAM DISTRICT, foothills of Mahendragiri, Burukhat area, on bark, 04.03.1986, D.D. Awasthi, G. Awasthi, R. Mathur & P. Srivastava 86-184 (LWG-AWAS).

REMARKS — *Phyllopsora chlorophaea* is distinguished by minute, lacinulate squamules and reddish brown prothallus. It is close to *P. mediocris* Swinscow & Krog in having reddish-brown prothallus and in cortex type, but the latter species differs in having medium sized, 0.3–1.0 mm wide squamules and in lacking lichen substances. *P. chlorophaea* is a pantropical species earlier reported from montane rainforest and humid woodland in Kenya, Peru, Mauritius, Tanzania, between altitudes of 600–1600 m (Broko 1991, Swinscow & Krog 1981, Timdal & Krog 2001, Timdal 2008). In India, the species is recorded from tropical to subtemperate areas in the states of Karnataka and Orissa, between altitudes of 200–800 m. It is a new to India.

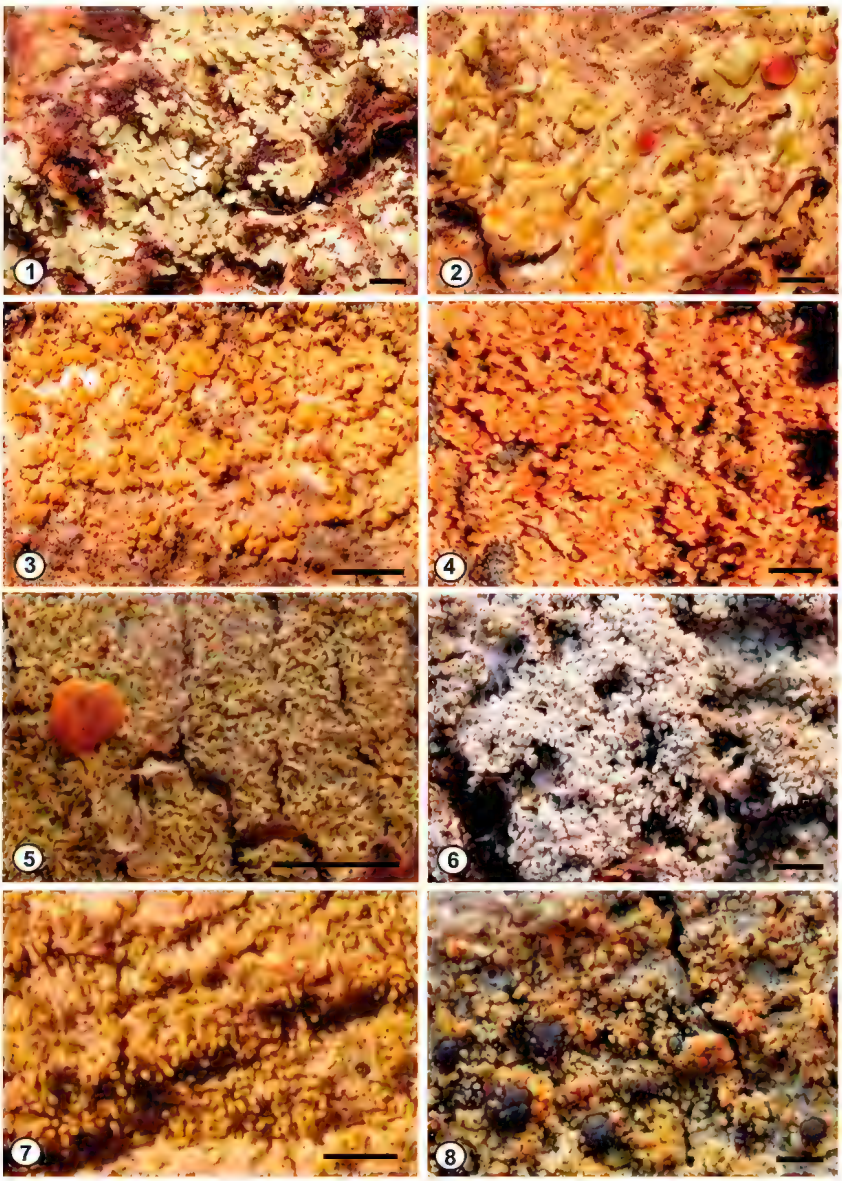
Phyllopsora confusa Swinscow & Krog, Lichenologist 13: 229 (1981). FIG. 5

Prothallus white to reddish brown. Thallus squamulose, adnate to ascending, overlapping, minute, 0.1–0.3 mm wide; upper surface glabrous, pale to green, plane to convex, finely pubescent along the margin. Isidia absent. Cortex of type 2, 10–20 µm thick; medulla containing crystals dissolving in K.

Apothecia common, up to 1.0 mm in diam., disc plane to convex, reddish-brown, margin slightly raised and glabrous; exciple colourless to pale brown; epihymenium hyaline; hymenium colourless; hypothecium reddish brown, containing crystals which dissolve in K. Ascospores hyaline, simple, narrowly ellipsoid, 8–11 × 2–3 µm. Pycnidia not seen.

CHEMISTRY — Thallus K–, C–, KC–, PD–; no lichen substance in TLC.

SPECIMENS EXAMINED — **INDIA: Assam**, NORTH CACHAR HILLS DISTRICT, Mahur area, on bark, 01.08.2005, D.K. Upreti & J. Rout 05-002958 (LWG), **Himachal Pradesh**, KANGRA DISTRICT, Macleodganj, alt. 1600–1800 m, on *Quercus leucotrichophora* bark, 13.05.2001, D.K. Upreti & S. Nayaka 01-75209 (LWG), **Karnataka**, SHIMOGA DISTRICT, Sagara, Holebagilu, alt. 623 m, on bark of *Knema attenuata*, 16.03.2001, S. Nayaka 01-022794 (LWG); Homnemardu Island, alt. 621 m, on bark of *Olea dioica*, 19.03.2001, S. Nayaka 01-66143 (LWG); Muppane, alt. 626 m, on bark of *Memecylon terminale* bark, 21.03.2001, S. Nayaka 01-66193, 01-66169 (LWG); Negiloni, alt. 710 m, on bark, 18.03.2001, S. Nayaka 01-107249 (LWG); 3km before Agumbe Ghat from Koppa, alt. 649 m, on evergreen forest, on bark, 14.01.2008, H.T. Lumbsch, D.K. Upreti & P.K. Divakar 1973/G (LWG); **Kerala**, ERNAKULAM DISTRICT, BS Thattekkad, alt. 430 m, on bark, 21.12.2006, B. Haridas 06-009600 (LWG); PALGHAT DISTRICT, Parambikulam Wildlife Sanctuary, alt. 610 m, on bark, 14.11.2006, B. Haridas 06-009823, 06-015954 (LWG); M.C.L. Mine area Walayar forest, alt. 300 m, on bark, 22.03.1985, D.D. Awasthi, R. Tiwari & R. Mathur 85.21 (LWG); SIRUVANI DISTRICT, alt. 810 m, on bark, 16.11.2006,



FIGURES 1–8. Habit. 1. *Phyllopsora albicans*; 2. *P. breviuscula*; 3. *P. catervisorediata* (holotype); 4. *P. chlorophaea*; 5. *P. confusa*; 6. *P. corallina var. subglaucella*; 7. *P. furfuracea*; 8. *P. himalayensis*. Bar = 2 mm

B. Haridas 06-009599 (LWG); TRIVENDRUM DISTRICT, ABP, Athirumala, Pathalamathy, alt. 1250 m, on bark, 25.04.2006, B. Haridas 06-009602 (LWG); **Uttarakhand**, PAURI DISTRICT, Pauri Kandoliya, on bark of *Cedrus deodara*, 19.06.2005, V. Shukla & Y. Joshi s.n. (LWG).

REMARKS — The most characteristic features of *P. confusa* are the minute, ascending squamules and the absence of lichen substances. It is close to *P. isidiotyla* in having reddish-brown prothallus and minute squamules but the latter species differs in having longer ascospores and fibrillose apothecia.

Phyllopsora confusa is a pantropical species earlier reported from montane rainforest and humid woodland in Kenya, La Réunion, Mauritius, and Tanzania between altitudes of 130–2000 m (Brako 1991, Swinscow & Krog 1981, Timdal & Krog 2001). In India, the species is recorded from tropical to temperate areas in the states of Assam, Himachal Pradesh, Karnataka, Kerala, and Uttarakhand and occurs between altitudes of 300–1800 m. It is new to India.

Phyllopsora corallina var. ***subglaucella*** G.K. Mishra, Upreti & Nayaka, var. nov.
MYCOBANK MB 518499 FIG. 6

Thallus squamulosus; squamulae ascendentes, elongatae, luteo-virescentes. Apothecia convexa, badio-fusca. Ascosporae ellipsoideae.

HOLOTYPE INDIA: Uttarakhand, PITHORAGARH DISTRICT, on way to Thalkedar Temple, alt. 1900–2400 m, on bark, 27.06.1983, D.K. Upreti 212878 (LWG).

ETYMOLOGY: From the similarity to var. *glaucella*.

Prothallus thin, white to reddish. Thallus squamulose, closely adnate, rounded to elongate, sometimes ascending, 0.1–0.3 mm wide; upper surface glabrous, pale green to yellowish, plane to convex, sometimes fibrillose at the margin, isidiate. Isidia cylindrical, thick, commonly bulbate at the base. Cortex of type 1–2, 5–8 µm thick; medulla containing crystals dissolving in K.

Apothecia common, up to 1.5 mm in diam.; disc plane to convex, brown; margin slightly raised. Exciple colourless; epihymenium hyaline; hymenium colourless; hypothecium colourless to pale brown. Ascospores hyaline, narrowly ellipsoid, 4–8 × 1–2 µm. Pycnidia common, yellow to brown, immersed in thallus; conidia rod-shaped, straight 6–15 × 1–2 µm.

CHEMISTRY — Thallus K+ yellow, C–, KC–, PD–; atranorin in TLC.

REMARKS — *Phyllopsora corallina* var. *subglaucella* is distinguished by the cylindrical to bulbate base of the isidia, cortex type 1–2 and presence of atranorin. It is close to the neotropical *P. corallina* var. *glaucella* (Vain.) Brako in having bulbate isidia, but the latter variety differs in having a cortex of type 2 and in containing different lichen substances, mainly vicanicin (Brako 1991).

This species is known from its type locality in the Western Himalayas, where it was found growing on bark of *Quercus leucotrichophora* and *Cedrus deodara* in moist and humid forest between altitudes of 1900–2500 m.

Additional Specimens examined — **India: Uttarakhand**, Bageshwar district, Dhakuri to Khati (en route to Pindari), alt. 2590 m, on bark, 20.05.1950, D.D. Awasthi & A.M. Awasthi 683, 676, 767 (LWG-AWAS); Loharkhet, Pindari Glacier, alt. 1760 m, on bark, 09.05.2007, S. Joshi & Y. Joshi 07-010710 (LWG); Pithoragarh district, Chandak Forest, alt. 1550 m, on *Quercus leucotrichophora*, May 2006, S. Bhatt, 06-009206, 06-010866 (LWG); Gangoli Hat, Hoat Kali Sacred Grove, on *Cedrus deodara*, H. Singh s.n. (LWG); Gori-ganga catchment, East Ghandhura, alt. 1600 2000 m, on bark, 03.10.2002, V. Pant 02000876, 02000880 (LWG); Narain Nagar, alt. 1500 m, on bark, 11.05.1977, O.P. Arora s.n. (LWG); Thakala Forest, alt. 1800m, on bark, 29.10.2002, V. Pant 02000673 (LWG).

Phyllopsora furfuracea (Pers.) Zahlbr., in Engler & Prantl, Nat. Pflanzenfam. 1(1*): 138 (1905). Fig. 7

≡ *Lecidea furfuracea* Pers., in Gaudichaud, Voy. Uran.: 192 (1827).

Prothallus well developed, thin, white. Thallus squamulose; squamules closely adnate, isodiametric, minute, 0.1–0.3 mm wide; upper surface glabrous, yellow green to green, rarely pubescent at margin, isidiate. Isidia common, simple, cylindrical, glabrous, rarely branching, sometimes arising directly from prothallus. Cortex of type 2, 7–10 µm thick; medulla containing crystals dissolving in K.

Apothecia common, up to 0.5–1.0 mm in diam.; disc plane to convex, brown, sometimes fibrillose at base; margin indistinct, paler than the disc. Exciple colourless to pale brown; epihymenium hyaline; hymenium and hypothecium pale brown throughout, the latter containing crystals which dissolve in K. Ascospores hyaline, simple, narrowly ellipsoidal, 5–6(–7) × 2–3 µm. Pycnidia not seen.

Chemistry — Thallus K–, C–, KC–, PD–; furfuraceic acid in TLC.

Specimens examined — **India: Kerala**, Palghat district, Parambikullam, Wildlife Sanctuary, alt. 450 m, on bark, 14.11.2006, B. Haridas, 06-009609 (LWG); Thirssur, Sholayar Range, Mukkanipara, alt. 450 m, on bark, 28.10.2006, B. Hridas 06-009609 (LWG); **Uttarakhand**, Pauri district, Srinagar, Dhari Devi Temple, on *Mangifera indica*, 26.06.2005, V. Shukla & Y. Joshi 05-005278 (LWG); Khirshu, on *Quercus*, 18.06.2005, V. Shukla & Y. Joshi 05-005103 (LWG); Pithoragarh district, Chandak Forest, alt. 1550m, on *Quercus leucotrichophora*, May 2006, S. Bhatt, 06-010888 (LWG); Ginni band, on bark, 30.10.2009, D.K. Upreti, S. Joshi, H. Rai, R. Khare, G.K. Mishra & A. Dwivedi 09-012601 (LWG); Munsiyari, Khuliya Top, alt. 2700-3000 m, on bark, 31.10.2009, D.K. Upreti, S. Joshi, H. Rai, R. Khare, G.K. Mishra & A. Dwivedi 09-012602 (LWG).

Remarks — *Phyllopsora furfuracea* is characterized by minute squamules and isidia directly attached to the prothallus. It is close to *P. nemoralis* in having usually white prothallus and minute squamules but the latter species differs in having smaller isidia and containing argopsin and atranorin. Two other species *P. dolichospora* Timdal & Krog and *P. furfuracea*, are close to *P. furfuracea* in chemistry but they are morphologically different.

The species is a pantropical species earlier reported from montane rainforest and humid woodland of Australia, Belize, Brazil, Colombia Cuba, Cuzco, Dominican Republic, East Africa, Ethiopia, Ecuador, French Guiana, Ivory Coast, Jamaica, Java, Kenya, La Réunion, Loreto, Madagascar, Mexico, Peru, Tanzania, United States, and Venezuela from sea level to an altitude of 2400 m (Brako 1991, Swinscow & Krog 1981, Timdal & Krog 2001, Timdal 2008). It is a new record for the Indian mycota and reported from the states of Kerala and Uttarakhand between altitudes of 300–2500 m.

Phyllopsora himalayensis G.K. Mishra, Upreti & Nayaka, **sp. nov.** FIG. 8

MYCOBANK MB 518500

Thallus squamulosus; squamulae ascendentes, elongatae, luteo-virescentes. Apothecia convexa, badio-fuscescentia. Ascosporae ellipsoideae vel fusiformes.

HOLOTYPUS INDIA: Himachal Pradesh, KULLU DISTRICT, Great Himalayan National Park, Shilt, alt. 2800 m, on bark, 04.11.2002, S. Nayaka & R. Srivastava 02-001037 (LWG).

ETYMOLOGY: From the Himalayas, the type locality.

Prothallus thin, white. Thallus squamulose; squamules closely adnate, rounded to elongate, ascending to overlapping, crenulate to incised, 0.1–0.5 mm wide; upper surface glabrous, pale green to yellow, plane to convex, pubescent along the margin, isidiate. Isidia mostly globular. Cortex of type 1–2, 7–10 µm thick, with scattered crystal dissolving in K; medulla containing crystals dissolving in K.

Apothecia common, up to 2.0 mm in diam.; disc plane to convex, brown to dark brown; margin raised. Exciple colourless; epihymenium hyaline to pale yellow; hymenium colourless; hypothecium colourless to pale yellow, containing crystals which dissolve in K. Ascospores hyaline, simple, narrowly ellipsoid to fusiform, 5–10 × 1–2.5 µm. Pycnidia common, orange to brown, immersed in the thallus; conidia rod-shaped, straight, 6–12 × 1–1.5 µm.

CHEMISTRY — Thallus K+ yellow, C–, KC–, PD–; atranorin in TLC.

ADDITIONAL SPECIMENS EXAMINED **INDIA: Himachal Pradesh**, KULLU DISTRICT, Great Himalayan National Park, Shilt, alt. 2800 m, on bark, 04.11.2002, S. Nayaka & R. Srivastava, 02-001152, 02-001113, 02-001077 (LWG); Pardi, alt. 3140 m, on bark, 05.11.2002, S. Nayaka & R. Srivastava, 02-001244 (LWG); **Uttarakhand**, BAGESHWAR DISTRICT, Dhakuri to Khati, en route to Pindari Glacier, alt. 2210–2734 m, on bark, 20.05.1950, D.D. Awasthi & A.M. Awasthi, 683 (LWG-AWAS); 12.05.2007, S. Joshi & Y. Joshi, 07-008931 (LWG); Tehri Garhwal, above Jamnotri, alt. 3657 m, on bark of *Quercus* tree, 22.06.1951, D.D. Awasthi 904 (LWG-AWAS).

REMARKS — *Phyllopsora himalayensis* is distinguished by the rounded to elongate, convex, pale green to yellow squamules, the globular isidia, and the colourless to pale yellow hypothecium. The new species is close to *P. kalbii* in

having globular isidia and dark brown apothecia, but *P. himalayensis* differs in having a cortex of type 2 and in lacking lichen substances.

Phyllopsora himalayensis occurs in temperate areas of the Himalayas in Himachal Pradesh and Uttarakhand between alt. 1500–3140 m. It grows on rough bark trees in moist and humid forests.

Phyllopsora isidiotyla (Vain.) Riddle, Mycologia 15: 81. (1923). FIG. 9
≡ *Lecidia isidiotyla* Vain., Etud. Lich. Bresil 2: 49 (1890).

Prothallus thick pale brown. Thallus squamulose; squamules adnate, granular to elongate, minute, 0.1–0.3 mm wide; upper surface glabrous, yellowish-green, fibrillose at the margin, isidiate. Isidia abundant, thick, cylindrical, rarely branched. Cortex thin, gelatinous, to 5 µm thick; medulla containing crystals dissolving in K.

Apothecia common, up to 1.0 mm in diam.; disc plane to convex, yellow-brown to brown; margin raised. Exciple pale yellow; epihymenium hyaline to pale yellow; hymenium golden yellow; hypothecium pale yellow-brown, containing crystals which dissolve in K. Ascospores hyaline, simple, ellipsoid to fusform, 6–11 × 1–2 µm. Pycnidia not seen.

CHEMISTRY — Thallus K+ yellow, C–, KC–, PD–; atranorin in TLC.

SPECIMENS EXAMINED — **INDIA: Assam**, NORTH CACHAR HILLS DISTRICT, Haflong, on bark, 01.08.2005, D.K. Upreti & J. Rout 05-002963 (LWG); **Bihar**, PACHIM CHAMPARAN DISTRICT, Don Hills, Devatapatta, on bark, 20.02.1995, D.K. Upreti & J. Tandon 213288 (LWG); **Himachal Pradesh**, CHAMBA DISTRICT, in and around Khajiar, alt. 2000 m, on bark, 15.05.2001, D.K. Upreti & S. Nayaka 01-75441 (LWG); **Karnataka**, SHIMOGA DISTRICT, Sagara, Moolagadde, alt. 600 m, on bark of *Calycopetris floribunda*, 22.3.2001, S. Nayaka 01-104966 (LWG); **Kerala**, PALGHAT DISTRICT, Sailent valley National Park, alt. 810 m, on bark, 16.11.2006, B. Haridas, 06-007945 (LWG); **Sikkim**, SOUTH SIKKIM DISTRICT, 20 km before Temi tea, state, alt. 1500 m, on bark, 31.03.2001, D.K. Upreti & S. Chatterjee 01-26673 (LWG); SOUTH SIKKIM DISTRICT, Narnchi 15 km towards Jagthang, alt. 1000 m, on bark, May 2006, D.K. Upreti & S. Chatterjee 219565 (LWG); **Tamil Nadu**, PALNI HILLS, Perumal to Palni road side, via short cut road, alt 1524 m, on bark, 15.12.1970, K. P. Singh 70.951 (LWG-AWAS); **Uttarakhand**, PAURI DISTRICT, near Kiyonkaleshwar Temple, on *Pinus*, 19.06.2005, V. Shukla & Y. Joshi 05-005449, 05-005443 (LWG); PITHORAGARH DISTRICT, Chandak Forest, alt. 1500 m, on *Quercus leucotrichophora*, May 2006, S. Bhatt 06-001088/B (LWG); Gori-ganga catchment, Thakala forest, alt. 1400 m, on bark of *Madhuca longifolia*, 29.10.2002, V. Pant 02000616 (LWG); Saurlekh forest area, Near Microwave Station, alt. 2700 m, on bark, 30.09.1990, D.K. Upreti & G.N. Hariharan 202253 (LWG); on way to Thalkedar temple, alt. 1900 2400 m, on bark, 27.06.1993, D.K. Upreti 212849/B (LWG).

REMARKS — *Phyllopsora isidiotyla* is distinguished by abundant isidia and granular squamules. It is close to *P. furfuracea* in having minute squamules and thick isidia, but the latter species contains furfuraceic acid.

The species is earlier reported from Brazil, Costa Rica, Venezuela, and United States, from sea level to an altitude of 3000 m (Brako 1991). In India

it is known from tropical to temperate areas in the states of Assam, Himachal Pradesh, Karnataka, Kerala, Sikkim, Tamil Nadu, and Uttarakhand, between altitudes of 600–2700 m. It is new to the Indian lichen biota.

Phyllopsora kalbii Brako, Flora Neotropica Monograph 55: 51 (1991). FIG. 10

Prothallus thin, white to pale brown. Thallus squamulose; squamules closely adnate to overlapping, crenulate to incised, 0.1–0.3 mm wide; upper surface glabrous, pale green, convex, pubescent along the margin, isidiate. Isidia mostly globular. Cortex of type 2, 5–17 µm thick; medulla containing crystals dissolving in K.

Apothecia common, up to 2.0 mm in diam.; disc plane to convex, brown to black; margin raised. Exciple pale brown; epihymenium hyaline; hymenium pale yellow; hypothecium colourless to pale yellow-brown, containing crystals which dissolve in K. Ascospores hyaline, simple, narrowly ellipsoid to fusiform, 5–12 × 2–3 µm. Pycnidia yellowish; conidia straight, 4–6 × 1 µm.

CHEMISTRY — Thallus K–, C–, KC–, PD–; no lichen substance in TLC.

SPECIMENS EXAMINED — **INDIA: Madhya Pradesh**, DINDORI DISTRICT, 13 km, before Kabir from Chauradader, alt. 640 m, on *Shorea robusta* tree trunk, 06.07.2005, D.K. Upreti, S. Nayaka & Satya 05-005704 (LWG); Around Jagatpur Forest Rest House, alt. 640 m, on *Shorea robusta* tree trunk, 05.07.2005, D.K. Upreti, S. Nayaka & Satya 05-005578 (LWG); Tarwartola near to Chauradeder, alt. 640 m, on *Shorea robusta* tree trunk, 06.07.2005, D.K. Upreti, S. Nayaka & Satya 05-005662, 05-005882/B (LWG).

REMARKS — *Phyllopsora kalbii* is distinguished by the globular isidia, mostly rounded, convex squamules, and brown to black apothecia. It is close to *P. corallina* var. *santensis* (Tuck.) Brako in having globular to cylindrical isidia and dark brown apothecia, but the latter taxon differs in chemistry (mainly argopsin).

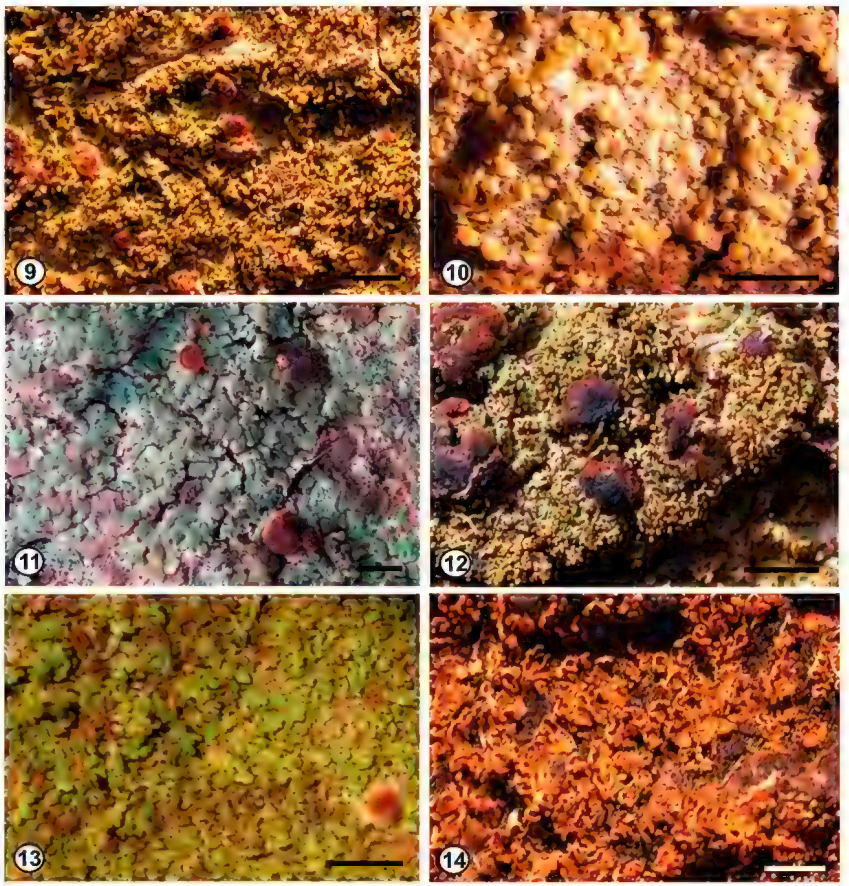
The species is a pantropical species earlier reported from Brazil, Dominican Republic, Kenya, Tanzania, and United States between altitudes of 300–2400 m (Brako 1991). In India, *P. kalbii* is known from tropical areas in the state of Madhya Pradesh, between altitudes of 300–650 m. It is new to the Indian lichen biota.

Phyllopsora mauritiana (Taylor) Gotth. Schneid., Biblioth. Lichenol. 13: 177 (1980, '1979'). FIG. 11

≡ *Lecidea mauritiana* Taylor, London J. Bot. 6: 151 (1847).

Prothallus thick, reddish-brown. Thallus squamulose; squamules closely adnate, overlapping, mostly isodiametric, 0.3–1.0 mm wide; upper surface glabrous, green, plane to convex, fibrillose at the margin. Isidia absent. Cortex of type 2, 8–20 µm thick; medulla containing crystals dissolving in K.

Apothecia common, up to 1.0 mm in diam.; disc plane to convex, pale brown



FIGURES 9–14. Habit. 9. *Phyllopsora isidiotyla*; 10. *P. kalbii*; 11. *P. mauritiana*; 12. *P. nemoralis*; 13. *P. subcrustacea*; 14. *P. swinscowii*. Bar = 2 mm

to brown; margin slightly raised and sometimes fibrillose. Exciple colourless to brown; epihymenium hyaline; hymenium hyaline to yellowish; hypothecium golden yellow, containing crystals which dissolve in K. Ascospores hyaline, simple, bacilliform, narrowly ellipsoid to fusiform, 6–9 × 1–2 µm. Pycnidia yellowish; conidia straight, 4–8 × 1 µm.

CHEMISTRY — Thallus K–, C–, KC–, PD–; no lichen substances in TLC.

SPECIMENS EXAMINED — **INDIA: Kerala**, PALGHAT DISTRICT, Siruvani, alt. 810 m, on bark, 16.11.2006, B. Haridas 06-009686, 06-009824 (LWG); **Tamil Nadu**, PALNI HILLS, Kodaikanal road, on way to Gumparai, alt. 914 m, on bark, 23.12.1970, K.P. Singh 70.1268 (LWG-AWAS); Shola near 18th mile of Kodaikanal road, alt. 1066 m, G. Foreau, S. J. 4141 (LWG-AWAS).

Remarks — The most characteristic feature of *P. mauritiana* is large, adnate, mostly isodiametric squamules, and golden yellow hypothecium. It is close to *P. breviuscula* in squamule and prothallus characters, but the later species forms thicker, elongated lobes.

Phyllopsora mauritiana was earlier reported from humid submontane forest in Tanzania and Mauritius between altitudes of 600–1500 m (Timdal & Krog 2001). In India it is recorded from tropical to temperate areas in the state of Kerala and Tamil Nadu between altitudes of 800–1066 m. It is a new species for the country.

Phyllopsora nemoralis Timdal & Krog, Mycotaxon. 77: 85 (2001). Fig. 12

Prothallus thin, reddish brown. Thallus squamulose; squamules adnate, granular to elongate, minute, 0.1–0.3 mm wide; upper surface glabrous, green, fibrillose at the margin, isidiate. Isidia abundant, thin, short, cylindrical, rarely branched. Cortex of type 1–2, 10–15 µm thick; medulla containing crystals dissolving in K.

Apothecia common, up to 2.0 mm in diam.; disc plane to convex, dark brown to black; margin raised. Exciple colourless; epihymenium hyaline; hymenium colourless; hypothecium pale brown, containing crystals which dissolve in K. Ascospores hyaline, simple, ellipsoid to fusform, 6–11 × 1–2 µm. Pycnidia not seen.

Chemistry — Thallus K+ yellow, C–, KC–, PD–; containing ± argopsin and atranorin in TLC.

Specimens examined — **India: Assam**, North Cachar Hills district, Hoflong, on bark, 01.08.2005, D.K. Upreti & J. Rout 05-003568 (LWG); Mahur, on bark, Sept. 2005, D.K. Upreti & J. Rout 05-009945/A (LWG).

Remarks — *Phyllopsora nemoralis* is characterized by the thin, small, short isidia and the colourless hymenium. It is close to *P. halei* (Tuck.) Zahlbr., in having a reddish brown prothallus and in the presence of atranorin, but that species differs in having thick, erect isidia and a dark reddish brown hypothecium. The other isidiate species, *P. isidiotyla,* differs in cortex type, while *P. furfuracea* contains furfuraceic acid.

Phyllopsora nemoralis was earlier known from woodland in Tanzania, La Réunion and South Africa between altitudes of 1550–2010 m (Timdal & Krog 2001). In India it is recorded from temperate areas in the state of Assam at an altitude of 1014 m. The species is new to the Indian lichen biota.

Phyllopsora subcrustacea (Malme) Brako, Mycotaxon 35(1): 15, 1989. Figure 13

≡ *Lecidea corallina* var. *subcrustacea* Malme, Ark. Bot. 28A(7): 47. 1936.

Prothallus thick, reddish brown to red. Thallus squamulose; squamules first adnate, later ascending, overlapping, minute 0.1–0.3 mm wide; upper surface

glabrous, yellow-green to green, plane to convex, finely pubescent along the margin. Isidia absent. Cortex of type 2, 8–20 µm thick, medulla containing crystals dissolving in K.

Apothecia common, up to 1.5 mm in diam.; disc plane to convex, reddish-brown; margin slightly raised and fibrillose. Exciple colourless to pale brown; epihymenium hyaline; hymenium colourless to pale yellow; hypothecium golden yellow, containing crystals which dissolve in K. Ascospores hyaline, simple, narrowly ellipsoid to fusiform, 12–21 × 2–3 µm. Pycnidia not seen.

CHEMISTRY — Thallus K–, C–, KC–, PD–; no lichen substances in TLC.

SPECIMENS EXAMINED **INDIA: Karnataka**, SHIMOGA DISTRICT, Sagara to Talgappa, Ulanhalli, alt. 697 m, on bark in evergreen forest, 16.02.2008, H.T. Lumsch, D.K. Upreti & P.K. Divakar 19742/0/2 (LWG); Sagara, Muppane, alt. 626 m, on bark, 21.03.2001, S. Nayaka 01-66191 (LWG); Shavauti River Basin, Holebagilu, alt. 650 m, on bark of *Diospyros crumenata*, 16.03.2001, S. Nayaka 01-222798, 01-22276 (LWG); **Kerala**, IDUKKI DISTRICT, Adimali Forest range, Chekuthon Mukku, alt. 750 m, on *Turpinia malabarica* bark, 16.02.2006, B. Haridas 06-009606 (LWG); PALGHAT DISTRICT, Parambikulam Wildlife Sanctuary, alt. 800 m, on bark, 14.11.2006, B. Haridas 06-009603 (LWG).

REMARKS — *Phyllopsora subcrustacea* is characterized by the minute, adnate to ascending squamules and the fibrillose apothecia. It is close to *P. manipurensis* in having adnate squamules and in the absence of lichen substances but that species differs in having small ascospores 5–10 × 2–2.5 µm and a white prothallus.

The species was earlier reported only from Paraguay (Brako 1991). In India this species is known from tropical areas in the states of Karnataka and Kerala between the altitudes of 300–800 m. It is new for the country.

Phyllopsora swinscowii Timdal & Krog, Mycotaxon 77: 88 (2001). FIG. 14

Prothallus thick, red to reddish brown. Thallus squamulose; squamules adnate, overlapping, isodiametric, scattered when young, slightly imbricate, crenulate to incised, 0.3–1.0 mm wide; upper surface glabrous, greenish to yellowish-brown, sometimes pubescent along the margin, isidiate. Isidia common, simple, cylindrical, medium thick, attached to the margin of the squamules. Cortex of type 1–2, 5–10 µm thick; medulla containing crystals dissolving in K.

Apothecia common, up to 1.5 mm in diam.; disc plane to convex, pale brown to brown; margin slightly raised. Exciple colourless; epihymenium hyaline; hymenium golden yellow; hypothecium colourless to pale yellow-brown, containing crystals which dissolve in K. Ascospores hyaline, simple, narrowly ellipsoid to fusiform, 5–8 × 1–2 µm. Pycnidia not seen.

CHEMISTRY — Thallus K–, C–, KC–, PD+ orange; methyl 2,7-dichloropsoromate and methyl 2,7-dicholoronorpsoromate in TLC.

SPECIMENS EXAMINED **INDIA: Karnataka**, SHIMOGA DISTRICT, Sagara, Mattikoppa, alt. 603 m, on bark of *Holigarna* sp., 17.03.2001, S. Nayaka, 01-67383, 01-67390 (LWG);

Hubse, alt. 594 m, on bark of *Diospyros crumenata,* S. Nayaka 01-222743, 01-67393/B (LWG); **Kerala**, IDUKKI DISTRICT, Pariyar Tiger, Reserve, Thekkady, Cheveloda, on bark, 22.03.2006, B. Haridas, 06-007969 (LWG).

REMARKS — *Phyllopsora swinscowii* is characterized by the PD+ orange reaction and the red, thick prothallus. It is close to *P. africana* Timdal & Krog, and *P. martinii* Swinscow & Krog in having a PD+ orange reaction but both those species contain different lichen substances.

The species has earlier been reported from woodland in East Africa, Kenya, Mauritius, and Tanzania between altitudes of 300–1900 m (Timdal & Krog 2001). In India it is recorded from tropical areas in the states of Karnataka and Kerala, between altitudes of ca. 100–600 m. It is a new species for the country.

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Studies in lichens and lichenicolous fungi: 7. More notes on taxa from North America

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Abstract— *Acarospora complanata*, *Fellhaneropsis myrtillicola*, and *Lecanora stramineoalbida* are reported new for North America north of Mexico. *Acarospora superfusa* is confirmed as occurring in North America. *Biatorella rappii* is placed in synonymy with *Ramonia microspora*.

Key words— Appalachian Mountains, Magnusson, Sonoran Desert, SE coastal plain.

1. ***Acarospora complanata*** H. Magn., Svensk. Bot. Tidskr. 18: 332. 1924.

Type: France. Provence-Alpes-Côte D'azur: Var Dist., Massif volcanique de la Courtine, pres Ollisules, 1923, *de Crozals* (hb. B. de Lesd.[n.v.-presumed destroyed], holotype; UPS! isotype).

Acarospora complanata was described from France (Magnusson 1924) and Magnusson recognized it as occurring in Africa and Mexico (Magnusson 1929, 1956). The species forms a brown areolate orbicular thallus with inconspicuous immersed apothecia and an effigurate margin with narrow lobes, a hymenium 80–90 µm high, paraphyses at mid-height mostly 2–2.5 µm in diameter, and a cortex that contains gyrophoric and lecanoric acids (KC+ pink). For a fuller description see Magnusson (1929).

Morphologically, the species does not appear related to *A. molybdina* (Wahlenb.) Trevis, *A. macrocyclos* Vain., or *A. wahlenbergii* H. Magn., which have thicker rugulose thalli, thinner paraphyses, and pseudo-lecanorine apothecia often in convex areoles. Instead, *A. complanata* appears morphologically more closely related to *A. trachyticola* (Müll. Arg.) Hue, currently known only from Peru (Magnusson 1929), from which it differs mainly in having a lower

hymenium (80–90 vs. 100–120 µm) and producing gyrophoric and lecanoric acids (absent in *A. trachyticola*).

The concept of *Acarospora complanata* as revised by Clauzade & Roux (Clauzade et al. 1981) differs from Magnusson's concept of the species (Magnusson 1929), which is the species concept used here. *Acarospora complanata* sensu Magnusson (1929) has a thallus that reacts KC+ pink (gyrophoric and lecanoric acids), but Clauzade & Roux (Clauzade et al. 1981) synonymized seven species with *A. complanata* that all lack these substances. These authors probably included more than one taxon in their concept of *A. complanata* and a revision of their material is needed.

Acarospora complanata was included in the checklist of lichens of North America north of Mexico (Egan 1987) because *A. obscura* H. Magn., which was reported by Magnusson (1956) from California, was one of the seven species synonymized with *A. complanata* by Clauzade & Roux (Clauzade et al. 1981). The type specimen of *A. obscura* was collected in Mexico and was in the herbarium of Bouly de Lesdain. It is presumed to be lost, but Magnusson (1929, 1956) treated *A. obscura* as a C– and KC–, species with a non-effigurate and non-orbicular thallus. The one specimen identified as *A. obscura* by Magnusson that was seen during the Sonoran Flora Project, was actually *A. veronensis* A. Massal. (Knudsen 2008).

Bruce McCune collected *A. complanata* s. str. in Montana just west of Pine Butte Swamp in the limestone steppes and we here report the species as new to North America north of Mexico. The McCune specimen is overgrowing another lichen, but the behavior appears competitive (for space) rather than parasitic.

North American specimen examined — **U.S.A. Montana. Teton Co.:** 47°50'N, 112°36'W, 1480 m, on limestone outcrops, viii.1985, *McCune 15165* (hb. McCune).

2. ***Acarospora superfusa*** H. Magn., Meddel. Göteborgs Bot. Trädgård 5: 65. 1930.

Type: U.S.A. New Mexico: Las Vegas, 2000 m., 1927, *Brouard* (hb. de Lesd. [n.v.-presumed destroyed], holotype; UPS!, isotype).

During the Sonoran Flora Project, a small number of specimens of *Acarospora superfusa* were seen, including the isotype. These were distinctly pruinose and broadly attached to the substrate because the specimens were predominately reduced or young and, consequently, Knudsen (2008) included *A. superfusa* within a broad concept of *A. veronensis*.

The study of additional, recently collected specimens led to the observation that unlike *Acarospora veronensis,* mature specimens of *A. superfusa* have larger pruinose areoles (0.5–1.5 vs. 0.2-0.5 µm) and one apothecium per areole (rather than up to six smaller apothecia) (Magnusson 1929, 1930; Knudsen 2008) The

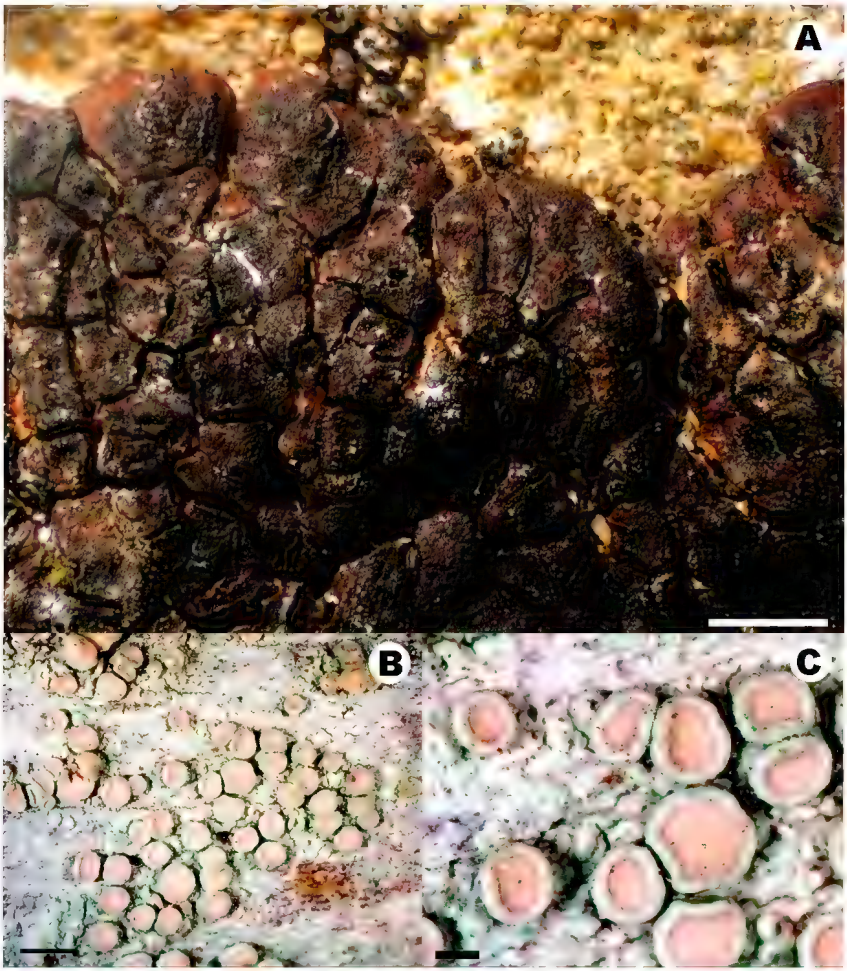

FIGURE 1. A, *Acarospora complanata* (McCune 15165, scale 1.0 mm). B-C, *Lecanora stramineoalbida* (Lendemer 20785, scale 1.0 mm and 0.25 mm respectively).

areoles of *A. superfusa* become gomphate (i.e., have an elongated mycelial base), and eventually lobulate and squamulose, developing a short stout stipe half the width of the squamule, rather than remaining broadly attached to the substrate as in *A. veronensis*. Otherwise, as observed by Magnusson (1930), in terms of cortical measurements, etc., *A. superfusa* is similar to *A. veronensis*.

Acarospora superfusa can also be confused with *A. nicolai* B. de Lesd. or pruinose specimens of *A. obpallens* (Nyl. ex Hasse) Zahlbr. However, both species produce gyrophoric and lecanoric acids. Thalli of *A. obpallens* further

differ by the presence of faveoles (Knudsen 2008), while *A. nicolai* differs in not becoming squamulose (Knudsen & Morse 2009). All three of the above species are sympatric from the Greater Sonoran Desert Region eastward to Oklahoma in the Great Plains.

Acarospora versicolor Bagl. & Carestia, which occurs in Europe and Siberia (Magnusson 1929; Clauzade et al. 1981), also appears to be closely related to *A. superfusa*, but has a higher hymenium on average (100–110 vs. 80–90 µm), a slightly thicker cortex, and narrower paraphyses (1.0–1.5 vs. 1.8–2.5 µm).

While revising specimens at the Farlow Herbarium (FH) a specimen of *Acarospora superfusa* from New Mexico was discovered that Magnusson had identified as *A. cinereoalba* (Fink) H. Magn. Based on this evidence it is likely that all the reports of *A. cinereoalba* from New Mexico by Magnusson (1930) are *A. superfusa*. *Acarospora cinereoalba* was originally described by Fink (1899) from Illinois and Minnesota as a variety of *A. cervina* (Ach.) A. Massal. We consider *A. cinereoalba* to be a pruinose morphotype of *A. veronensis*. While such pruinose specimens of *A. veronensis* are rare in North America they do occur, especially near waterfalls or on rocks were water settles and evaporates. Specimens of *A. superfusa* that are young or reduced and have not become gomphate or stipitate may be indistinguishable from pruinose forms of *A. veronensis*.

The protologue of *Acarospora superfusa* (Magnusson 1930) does not include an etymology. It is not known what Magnusson meant by the inclusion of "fusa" in the epithet. While he might have meant "fusca" (brown) alluding to a similarity to *Acarospora fusca* B. de Lesd., he nonetheless retained the original spelling 26 years later (Magnuson 1956) and we do the same here.

Specimens examined **Canada. Saskatchewan:** Rural Municipality of Waverley No. 44, Grasslands National Park, eroded bluff overlooking McGowan Creek Valley, on acidic rock, 49°03'54"N, 106°31'27"W, 899 m, 19.vi.2009, *Freebury 859* (hb. Freebury); eroded hilltop on mixed-grass prairie, on acidic sandstone, 898 m, 12.ix.2009, *Freebury 999* (hb. Freebury). **U.S.A. California. San Bernardino Co.:** San Bernardino Mountains, Clark Mountains, east edge of wash south of Pachalka Springs, 35°30'44" N, 115°37'17"W, 1548 m, on Hcl- boulder, rare, 11.x.2009, *Knudsen 11769 & Pietrasiask* (UCR). **San Diego Co.:** Anza Borrego Desert State Park, Plum Canyon, 33°06'28"N, 116°25'43"W, 688 m, on granite boulder in wash, rare, 22.ix.2005, *Knudsen 3665 & Marsden* (UCR). **Oklahoma. Greer Co.:** Quartz Mountain Arts & Conference Center, along New Horizon Trail, 34°53'35"N, 99°18'05"W, 482–588 m, on pink granite outcrops, 10.iv.2007, *Morse 15048 & Ladd* (KANU, NY, UCR).

3. ***Fellhaneropsis myrtillicola*** (Erichsen) Sérus. & Coppins, Lichenologist, 28(3): 199. 1996.

Type: Germany: Schleswig-Holstein, Lauenburg, im Sachsenwald bei Friedrichsruh, an *Vaccinium myrtillus* am Rande des Reviers Saupark, 2.xi.1924, *Erichsen* (HBG [n.v.], holotype).

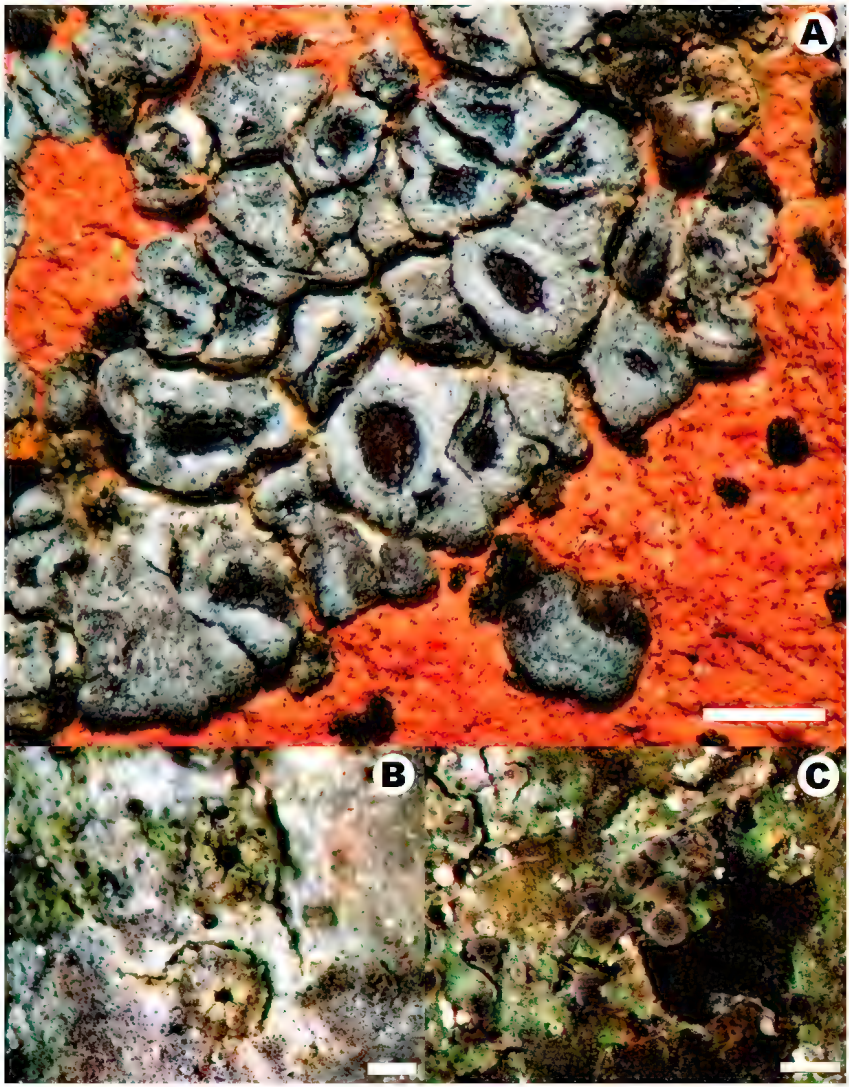

FIGURE 2. A, *Acarospora superfusa* (Freebury 999, scale = 0.5 mm). B, *Ramonia microspora* (Lendemer 20125, scale = 0.2 mm). C, *Fellhaneropsis myrtillicola* (Buck 50138, scale = 0.2 mm).

During the 2006 H.L. Bloomquist Foray, William Buck collected an interesting foliicolous lichen on *Rhododendron* leaves at the base of a waterfall at a high elevation site in the southern Appalachian Mountains. Foliicolous lichens

are not uncommon on evergreen leaves in high humidity microhabitats in this region; however they are only rarely collected. This collection remained unidentified until recently when Robert Lücking agreed to examine it and determined it to represent *Fellhaneropsis myrtillicola*. This is the first report of the species from North America and at his request we are publishing it here.

Fellhaneropsis Sérus. & Coppins resembles *Fellhanera* Vězda in most respects, differing in exciple type and in having filiform-sigmoid (vs. bacilliform) conidia (Sérusiaux 1996; Lücking 2008). Although *Fellhanera* species are common in the southern Appalachians, using the key provided by Harris & Lendemer (2009) none can easily be confused with *Fellhaneropsis* on account of the different conidia. *Fellhaneropsis myrtillicola* can be separated from other Pilocarpaceae in the region by its filiform-sigmoid conidia, 3-septate hyaline ascospores, and very small dark brownish-black convex immarginate apothecia. For a full description see Sérusiaux (1996). *Fellhaneropsis vezdae* (Coppins & P. James) Sérus. & Coppins has also been reported from western North America (Tønbserg 1997), however it differs from *F. myrtillicola* in having 5-septate ascospores.

Specimen examined — **U.S.A. North Carolina. Jackson Co.:** Nantahala National Forest, Panthertown Valley, vicinity of Schoolhouse Falls along Greenland Creek, 1190 m, hemlock-pine-hardwood forest with gneissic exposures, on *Rhododendron* leaves, 29.iv.2006, *Buck 50138* (NY).

4. ***Lecanora stramineoalbida*** Vain., J. Bot. 34: 35. 1896.

Type: Saint Vincent: Richmond Valley, *Elliott 239* (TUR-V 5582 [n.v.], holotype).

While collecting on Sapelo Island, Georgia, in December 2009, the first author collected an unusual *Lecanora* species with a dark (vs. hyaline) hypothecium on *Juniperus* in a dense coastal maritime forest. Using the revision of *Lecanora* species with dark hypothecia by Lumbsch et al. (1996) the collection keyed to *L. stramineoalbida*, a rare species from the West Indies. This is the first report of the species from North America. *Lecanora stramineoalbida* can be recognized by its corticolous habit, sessile apothecia, epihymenium with crystals that dissolve in K, brown K– hypothecium, and the production of 2'-0-methylperlatolic acid in addition to atranorin. For a full description refer to Lumbsch et al. (1996).

Specimen Examined **U.S.A. Georgia. McIntosh Co.:** Sapelo Island, behind Nanny Goat Beach, on *Juniperus* branch, 15.xii.2009, *Lendemer 20785* (NY).

5. ***Ramonia microspora*** Vězda, Folia Geobot. Phytotax., 1: 162. 1966.

Type: Argentina. Jujuy Prov.: Laguna de la Brea, 1901, *R.E. Fries* (S [n.v.], holotype)

Biatorella rappii Zahlbr., Ann. Mycol. 29: 82. 1931, **syn. nov.**
[non *Ramonia rappii* Vězda 1966].

Type: U.S.A. Florida. Seminole Co.: Mecca, xii.1923, on *Carpinus*, *Rapp* (W [n.v.], holotype; FLAS-27141!, isotype).

Ramonia microspora is a minute, easily overlooked, crustose lichen that is widespread throughout the Coastal Plain of southeastern North America. For a full description see Vězda (1966). It can be distinguished from other species of *Ramonia* using the key to the genus provided by Lendemer & Knudsen (2008). Recently Richard Harris pointed out that the type of *Biatorella rappii* is conspecific with *R. microspora*. Although *B. rappii* is the older name, the epithet "rappii" is preoccupied in *Ramonia* by *R. rappii* Vězda. Thus *B. rappii* should be placed in synonymy with *R. microspora*, and we do so here. A selection of specimens documenting the geographic distribution of this species in North America is provided below.

Specimens examined **U.S.A. Arkansas. Franklin Co.:** Ozark National Forest, Boston Mountain Ranger District, Shores Lake, 17.x.2005, *Harris 51759-A* (NY). **Georgia. Candler Co.:** Charles Harold TNC Preserve, 22.xii.2009, *Lendemer et al. 21756* (NY). **Pierce Co.:** Little Satilla Wildlife Management Area, 21.xii.2009, *Lendemer et al. 21515* (NY). **Louisiana. East Baton Rouge Parish:** Burden Research Plantation, 16.v.1979, *Tucker 18603-A* (NY). **Missouri. Butler Co.:** Big Cane Conservation Area, 23.x.2001, *Harris 45255* (NY). **North Carolina. Camden Co.:** Dismal Swamp State Park, 10.xii.2009, *Lendemer 20125 & Williams* (NY). **Carteret Co.:** Bogue Banks, Theodore Roosevelt Natural Area, 20.iii.2003, *Buck 43860* (NY). **Jones Co.:** Croatan National Forest, Catfish Lake South Wilderness, 17.iii.2003, *Buck 43723* (NY). **Pender Co.:** Holly Shelter Game Land, 18.iii.2003, *Buck 43757* (NY). **South Carolina. Aiken Co.:** Savannah River Bluffs Heritage Preserve, 13.iii.2010, *Harris 55994-A* (NY), *Lendemer 21954* (NY).

Acknowledgments

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Phyllactinia sebastianiae sp. nov. on *Sebastiania brasiliensis*

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ABSTRACT The new powdery mildew species *Phyllactinia sebastianiae* on *Sebastiania brasiliensis* (*Euphorbiaceae*) is described. This species was collected in Corrientes, Argentina, and is well characterized by conidiophores with twisted foot-cells and dimorphic conidia (lanceolate primary conidia and oblanceolate to ellipsoid-ovoid secondary conidia), indicating an anamorph belonging in the genus *Streptopodium*. Detailed descriptions and illustrations of both anamorph and teleomorph states of the new species are given, and it is compared with morphologically similar taxa.

KEY WORDS *Erysiphales*, taxonomy

Introduction

Recent collections of infected leaves of *Sebastiania brasiliensis* (*Euphorbiaceae*) from local forests of Corrientes, a northeastern province of Argentina, showed severe symptoms of powdery mildew. *Sebastiania brasiliensis* is a native tree of South American subtropical rain forests phytogeographically characterized as "Provincia Paranaense". The region has a warm and humid climate, with precipitations of about 1500 mm per year, more abundant in summer. The medium temperature of the year in this region is 20–21°C (Cabrera 1976). *S. brasiliensis* ("lecheron" or "palo de leche") grows wild in Corrientes in virgin forests, along path edges and ditches, or untilled landscapes. It is a species without timber value but is used as an ornamental due to its conspicuous waxy leaves.

The anamorphic genus *Oidium* Link. seems to be the predominant representative of *Erysiphales* on plants of the *Euphorbiaceae* in the "Paranaense" region. Many species (e.g., *Acalypha wilkesiana* Müll.-Arg., *Croton bonplandianus* Baill., *C. glandulosus* L., *Euphorbia heterophylla* L., *E. hirta* L. var. *hirta*, *E. tithymaloides* L., *Jatropha curcas* L., *J. breviloba* (Morong) Pax &

K. Hoffm., *Manihot esculenta* Crantz, *Microstachys hispida* (Mart.) Govaerts) are infected by *Oidium* spp. These unpublished data supplement the data given in Amano (1986), who listed only *Oidium* spp. and *Phyllactinia suffulta* (Rebent.) Sacc. on *Sebastiania* sp.

Other South American records of *Phyllactinia* Lév. refer to collections on *Erythrina* spp. (*Fabaceae*) and *Morus alba* L. (*Moraceae*) (Mazzanti & Cabrera 1985). Additional South American species (Braun 1987, Havrylenko 1995, Havrylenko et al. 2006) include *Phyllactinia adesmiae* Havryl. on *Adesmia* (*Fabaceae*), *P. ampulliformis* Havryl. on *Discaria* (*Rhamnaceae*), *P. antarctica* Speg. on *Ribes* (*Grossulariaceae*), and *P. chubutiana* Havryl. et al. on *Lycium* (*Solanaceae*) from Argentina and *P. caricaefolia* Viégas on *Carica* (*Caricaceae*) and *P. chorisiae* Viégas on *Chorisia* (*Bombacaceae*) from Brazil.

In this work, a new powdery mildew on *S. brasiliensis* is treated in detail and compared with allied species.

Materials & methods

During 2007 and 2008, leaves showing symptoms of powdery mildew were collected from adult plants in the field. All sites are situated in the province of Corrientes, Argentina (see MATERIAL EXAMINED).

Anatomical observations

The fungal fruiting bodies were carefully scraped from the leaf surfaces with moistened pointed dissection needles and mounted in distilled water. The specimens were then stained and embedded with lactophenol cotton blue (LB) (Gams et al. 1980). Penicillate cells were stained with lactofuchsin (LF) (Carmichael 1955) over a small flame (Shin & Lee 2002). Hyphae, conidiophores, and conidia were stripped from the leaf surfaces with clear adhesive tape and mounted in water, LB, or LF on a microscope slide with the fungal material uppermost. Dried herbarium material was first restored by boiling a small piece of infected leaf in a drop of lactic acid (Shin & La 1993) before being examined as described above. In order to observe the endophytic mycelium, the technique of leaf clearing of Liberato et al. (2005) was used. In each case, fifty measurements of structures of taxonomical value (× 400 magnification) were made, with the average given in parentheses.

CONIDIAL GERMINATION — Conidial germ tubes were observed by incubation on glass slides in damp chambers at room temperature and pressing a sporulating leaf surface directly onto a slide or dislodging conidia by tapping an infected leaf (Boesewinkel 1980, Cook & Braun 2009). In addition, the inoculation on the epidermis of onion scales was used (To-anum et al. 2005). In both cases, the conidia were incubated at 20°–25°C for 24 h until microscopic observation.

LIGHT MICROSCOPY — Micrographs were taken with a NIKON SC 102 light microscope and a camera Sony Cyber-shot 4.1 mpegmovie VX.

SCANNING ELECTRON MICROSCOPY — Fresh leaves with the most suitable areas with powdery mildew symptoms were selected for detailed study. Small pieces about 5 mm^2 were cut out and fixed for 48 h in FAA. The samples were washed, dehydrated, critical

point dried, and sputter coated with gold according to D'Ambrogio de Argüeso (1976). SEM preparations were observed in a JEOL 5800 LV microscope.

Taxonomy

Phyllactinia sebastianiae M.G. Cabrera & Vobis, **sp. nov.** FIGURES 1–6

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Phyllactiniae robiniae *morphologice valde similis, sed conidiophoris ad 287.5 µm longis, conidiis ad 120 × 28 µm, conidiis secundariis oblanceolatis vel ellipsoideis-ovoideis, truncis cellularum penicillatarum chasmotheciorum minoribus, 25 30 × 15 25 µm, simplicibus vel bifurcatis, ascis 2-sporis.*

TYPE: Argentina, Corrientes province, Santa Ana de los Guácaras, on living leaves of *Sebastiania brasiliensis* Spreng. (*Euphorbiaceae*), 6 Jun. 2007, M.G. Cabrera. (Collection N° 442; CTES 0591005, **holotype**; BCRU 05146, isotype).

ETYMOLOGY: derived from the name of the host genus, *Sebastiania*.

SYMPTOMS The symptoms of typical powdery mildew were restricted to the leaves of *Sebastiania brasiliensis* trees. Forming at first rounded to irregular patches of 0.5 to 1 cm in diameter, the fungal parasite developed typical mycelia on both sides of the leaves, predominantly on the adaxial surface (FIG. 1AB). The infections varied and were more abundant on mature than on young leaves. During mycelial development, the leaves changed in color from green to yellow and copper-colored. At a later stage, the most affected areas turned golden-brown (FIG. 1B). The leaves lost their flexibility, dried, and became fragile but kept their flat form. Extensively colonized leaves showed slight chlorosis and later developed a tan necrosis (FIG. 1AB).

The initial mycelia were white, causing a yellowing of the host tissue. Subsequently, the mycelia became denser and changed to yellowish or ash-colored by producing abundant conidia. At this stage, the fungus showed the typical powdery aspect. A hyperparasitic *Cladosporium* sp. frequently slowed down the development of the mycelium, finally destroying it, and darkening the surface of the leaves.

Throughout the year, the foliage of the trees of *S. brasiliensis* was attacked by the powdery mildew in an irregular manner. During the summer, from November to April, the mycelia were scarce, rather opaque, and shriveled. Only slight production of turgescent conidia could be observed. The more remarkable infections were detected during the cold and frosty winter months from May to August, but sometimes also through all seasons, especially on trees growing in shady habitats or in perennially humid environments. In severe attacks, the diseased plants appeared highly deteriorated with completely defoliated branches.

MYCELIUM Two types of mycelia developed: external and internal.

The ectophytic mycelium is amphigenous, at first delicate, later becoming dense, effuse at maturity, moderately thick and subpersistent, more compressed

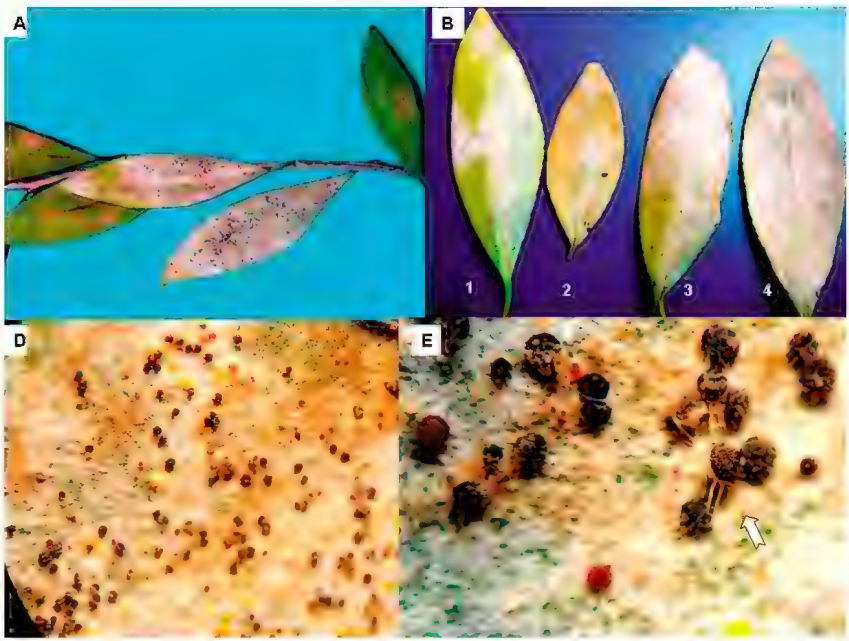

FIGURE 1. *Phyllactinia sebastianiae* aspect. A. Powdery mildew symptoms and occurrence of chasmothecia on *S. brasiliensis* leaves. B. Mycelia covering the adaxial surfaces (1 and 3), and abaxial surfaces (2 and 4). C. Group of immature and mature chasmothecia on mycelium. D. Chasmothecium with acicular appendages

with age. The mycelium is initially whitish, later yellow. Finally, the external mycelium covers the whole surface of the leaf (FIG. 1AB). The ectophytic hyphae are almost straight to sinuous, sometimes geniculate, hyaline, thin-walled, smooth and septate, branched, with long cells, 44(72.5)140 × 3.75(5.8)8 µm (FIG. 3CD). Appressoria are solitary, variable in shape, ranging from nipple-shaped, lobed to almost coralloid to hooked or slightly forked (FIGS. 3F 6E). The appressoria are generally not very abundant.

In addition, a typical endophytic mycelium is developed (FIG. 3C). In transverse sections of the leaf, hyaline, branched hyphae were observable within the host plant tissue (FIG. 5A). Globose haustoria formed terminally on short hyphal side branches (FIG. 5B). Ectophytic and endophytic mycelia are connected by hyphae emerging through the stomata (FIG. 3C).

CONIDIOPHORES The anamorphic structures are characterized by hyaline conidiophores, which arise directly from the external mycelium (FIG. 3D). They are 75(158)287 µm long and 5(6.78)8 µm wide. The basal septa are situated above the junctions with the supporting ectophytic hyphae, up to about 7.5 µm

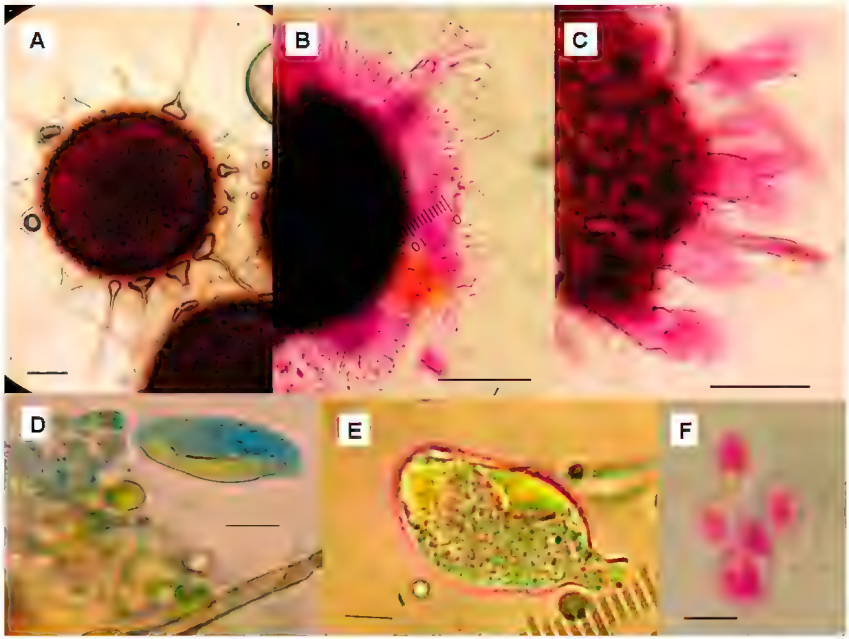

FIGURE 2. Characteristics of *Phyllacinia sebastianiae* conidia. AC. Primary conidia. DE. Secondary conidia. Germ tube development in B, C and E. (Bar: 25 µm)

(FIG. 6E). The long foot-cell, 37(158)250 × 5(6.78)8 µm, is twisted in 1–3 loops or sinuous at its base (FIG. 3D) and followed by 1 or 2 shorter cells (FIG. 6E).

CONIDIA The conidia are developed singly at the apex of the conidiophore (FIG. 6E). Sometimes two conidia are produced successively. They are one-celled and hyaline with thin walls. Young conidia are slightly rough, becoming rougher or slightly reticulate when mature (FIGS. 2DE 3E). In young conidia, the cytoplasm is hyaline with abundant vacuoles of different sizes (FIG. 2A). Oil drops can also be observed (FIG. 2B). Two types of conidia are produced (FIG. 6E). The primary conidia are lanceolate and apically pointed (FIGS. 2AC 4E), whereas the secondary conidia are more oblanceolate to ellipsoid-ovoid (FIGS. 2DE 3F). Primary and secondary conidia have similar sizes (57.5(78)120 × 14(24)28 µm).

CONIDIAL GERMINATION The germ tubes are usually produced in a subapical position, sometimes also laterally or at both apices. They are 70–95 µm long and develop unlobed or multilobed appressoria (FIGS. 2BCE 3F 6E). Under natural conditions, the germination rate for both primary and secondary conidia was moderate (70%). Laboratory assays demonstrated a lower germination rate, 23% for the incubation method on glass slides, and 29% for inoculation on onion scales.

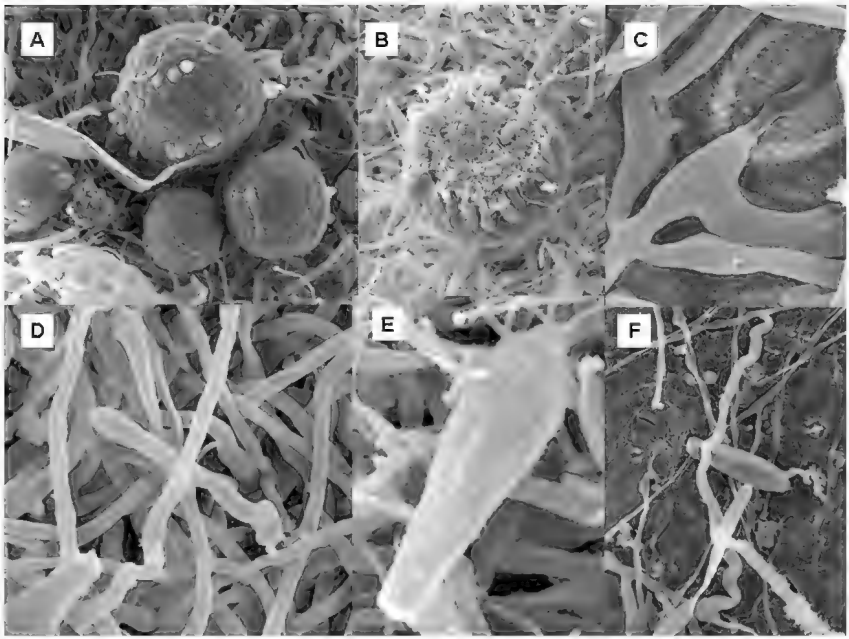

FIGURE 3. Teleomorphic structures of *Phyllactinia sebastianiae* (holotype). A. Anatomical structures of ascomata. B. Superficial vision of numerous filaments and penicillate cell. C. Peridial cells and basal penicillate cell D- Immature ascus and ascospores E. Pedicellate immature ascus. F. Immature ascospores. ((Bar: A,B, 50 µm; C,F. 25 µm; D. 15 µm; E. 12 µm)

CHASMOTHECIA The chasmothecial ascomata are gregarious or scattered on both surfaces of the leaves (FIG. 1AC). They are spherical when young (FIG. 4A), becoming later distinctly depressed at the lower side (FIG. 1D). The color of the initials of the fruiting bodies is white, changing to pale yellow and orange, and finally turns dark brown or nearly black when mature (FIG. 1CD). The well developed chasmothecia have diameter of 142(180)250 µm. The peridium consists of polygonal cells with thick and dark walls. (FIGS. 3A 4A 6A). They measure 6.25(10)12.5 µm in diameter.

APPENDAGES Each individual fruiting body presents two types of appendages: acicular appendages and penicillate cells.

The acicular appendages are arranged in equatorial position. Each chasmothecium bears 9(17)22 acicular appendages (FIG. 3A), which are rigid, hyaline, with thick walls, having a crystalline or vitreous appearance (FIG. 1D). Their bases are swollen, forming a 27.5(32.5)40 µm diam., globular structure, and the apexes are pointed (FIG. 6AC). The acicular appendages are variably long (107(195)312.5 µm), generally exceeding the diameter of the chasmothecium.

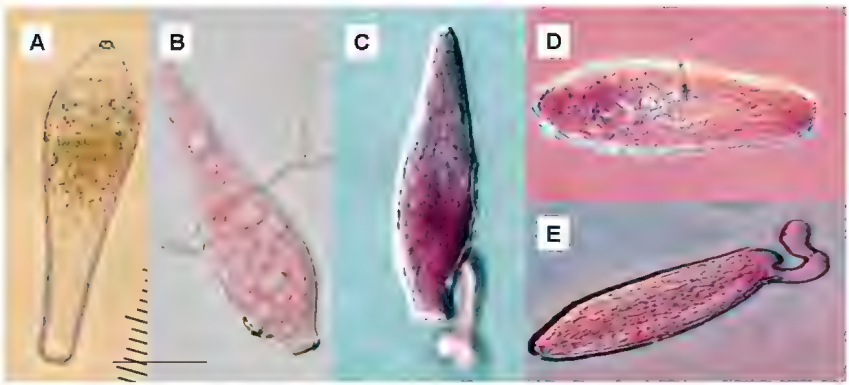

FIGURE 4. SEM Micrographs of *Phyllactinia sebastianiae*. A. Development of penicillate cells on chasmothecia. B. Young penicillate cells and acicular appendages of immature ascoma (500×). C. Hypha protruding from a stoma (2000×). D. Conidiophores and hyphae of external mycelium and conidiophores (1000×). E. Rough conidium outer surface (1700×). F. Germ tube with appressorium of secondary conidium (700×)

The penicillate cells, which are located on the upper side of the ascoma (FIGS. 3B 4B), are composed of a stem that bears free thin filaments (FIGS. 4B 6B) and is connected to the peridium and protrudes from the surface of the ascoma wall (FIG. 4C). The stem is roughly cylindrical, 25–30 × 15–25 µm, occasionally bifurcate in its upper part and extending into numerous, thin, 25–70 µm long filaments. The stem walls are thin and the filaments are embedded in a mucilaginous mass that covers completely the top of the chasmothecium (FIG. 3B).

ASCI AND ASCOSPORES Various specimens, collected from the same sites during different periods over two years, were investigated, but material with totally mature asci was never observed. The description is based on collections obtained in winter, the most favourable season for growth and development of reproductive structures. The asci are arranged in clusters inside the ascoma. They are broadly clavate to globoid, with well-developed pedicels (FIGS. 4DE 6D).

The ascus walls are thin and hyaline. The asci are 50–57.5 × 32.5–37.5 µm. It is estimated that about 10% of the asci produce ascospores under the above characterized natural conditions. A number of 2-3 ascospores per ascus perhaps can be observed. The ascospores are hyaline, ellipsoidal to ovoid (FIG. 6D), having a dense cytoplasm, which contains 2-3 yellow oil drops (FIGS. 2F). They are 25–37.5 µm long and 18–22.5 µm wide.

ADDITIONAL SPECIMEN EXAMINED: ARGENTINA. CORRIENTES PROVINCE, Santa Ana de los Guácaras, near National Route 12 (S 27°2'; W 58°23'), 4 June 2007, R.E. Álvarez N° 441 (CTES 0591004).

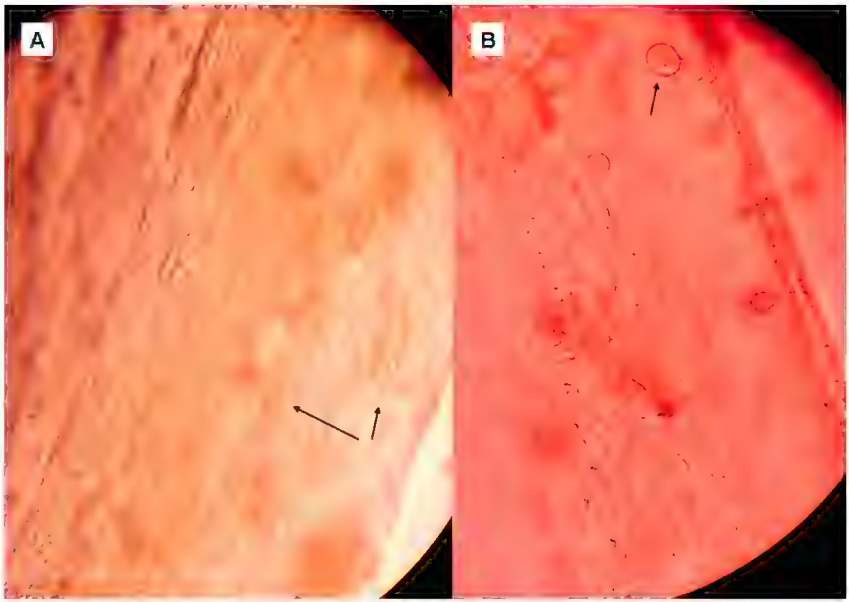

FIGURE 5. *Phyllactinia sebastianiae* A. Endophytic hyphae within *S. brasiliensis* leaf. B. Formation of haustoria.

Discussion

Although the median temperature of the year in the Paranaense phytogeographical region is 20–21°C (Cabrera 1976), in the province of Corrientes the temperature ranges from –2.8°C to 42.4°C (median temperature 26.8°C) (Sánchez et al. 2007). This thermic variation is important for its influence on chasmothecial maturity. During the growing season the fungus produces mainly the anamorphic stage. Ascospores were usually immature.

The fungus exhibits the typical anamorphic morphology of *Streptopodium* R.Y. Zheng & G.Q. Chen as circumscribed by Braun (1987), characterized by a hemiendophytic mycelium that enters the leaf tissue through stomata (FIG. 3C) and dimorphic conidia (FIG. 6E). The conidia outer walls are verrucose when viewed by SEM (FIG. 3E), and the conidiophores have sinuous bases (FIG. 3DF). Ascoma characters agree with those of the genus *Phyllactinia*.

Amano (1986) registered *Oidium* sp. and *Phyllactinia suffulta* on *Sebastiania* sp. However, the anamorph of *P. guttata* (Wallr.) Lév. (the current name for *P. suffulta*) belongs to *Ovulariopsis* Pat. & Har. (Takamatsu et al. 2008), which is characterized by straight conidiophores. The anamorph of the fungus we observed on *S. brasiliensis* does not belong to *Ovulariopsis*, and the teleomorph is not conspecific with *P. guttata* (Braun 1987). Furthermore, the conidiophore foot-cells are spirally twisted or sinuous. Several *Phyllactinia* spp. with twisted

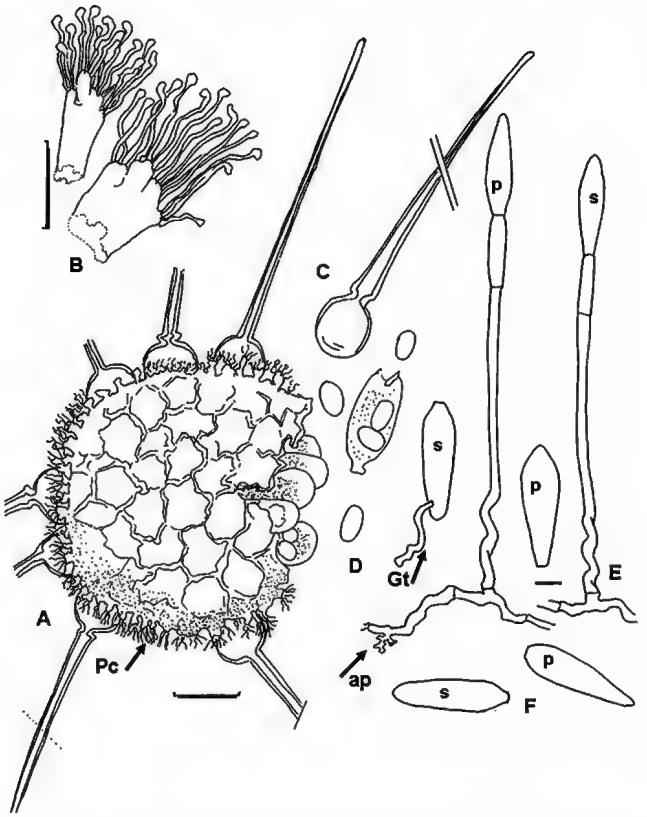

FIGURE 6. *Phyllactinia sebastianiae* (holotype). A. Chasmothecium with peridial cells and appendages. B. Numerous filaments on penicillate cells. C. Acicular appendage with swollen base. D. Pedicellate ascus and ascospores. E. Conidiophores with sinuous basal cells. F. Primary and secondary conidia. (Bar: A, 20 µm; B, 40 µm; C F, 20 µm)

conidiophore foot-cells and dimorphic conidia are known, but all of them inhabit hosts of unrelated families. *Phyllactinia robiniae* U. Braun & Yáñez-Morales is morphologically close to *P. sebastianiae* but differs in having shorter (70–130 µm) conidiophores, narrower (40–70 × 12–22 µm) clavate secondary conidia, penicillate cells with narrower stems (25–50 × 8–18 µm), several short terminal branchlets, and 2-spored asci (Braun & Yáñez-Morales 2009). *Phyllactinia erythrinae-americanae* Yáñez-Morales & U. Braun (Braun & Yáñez-Morales 2009) is distinguished by its conidiophores with basal septa elevated 10–35 µm above the junction with the supporting hypha. Furthermore, the twisted part of the conidiophores is usually confined to the portion below the basal septum. The *Phyllactinia* sp. on *Erythrina* spp. in Argentina (Mazzanti de Castañón &

Cabrera de Álvarez 1985) is morphologically rather close to the latter species but differs in having larger chasmothecia with a larger number of appendages. The taxonomy of these collections from Argentina is not yet clear (Braun & Yáñez-Morales 2009). *Phyllactinia caricaefolia* on *Carica papaya* in Brazil is also characterized by forming dimorphic conidia but this species is easily distinguishable from *P. sebastianiae* by its much smaller ascomata and distinctly clavate secondary conidia (Viégas 1944, Liberato et al. 2004). *Streptopodium caricae* Liberato & R.W. Barreto (Liberato et al. 2004) is probably the anamorph of *P. caricaefolia*. *Phyllactinia chorisiae* on *Chorisia speciosa* in Brazil is another species with dimorphic conidia, but the conidiophores are straight and the secondary conidia distinctly clavate (Liberato 2007). *Phyllactinia dalbergiae* Piroz. on hosts of the *Fabaceae* possesses conidiophores with twisted foot-cells, but the conidia are uniformly clavate (Mukerji 1968, Braun 1987).

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Two new species of *Lyophyllum* s.l. (*Basidiomycota, Agaricomycetes*) from La Palma (Canary Islands, Spain)

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Abstract — Two new species in the genus *Lyophyllum* collected in La Palma (Canary Islands), *L. fuscobrunneum* and *L. impudicum*, are here described and taxonomically delimited based upon morphological data. For each species detailed descriptions, microscopical drawings, and plates are provided.

Key words — *Agaricales, Lyophyllaceae,* taxonomy, biodiversity

Introduction

The present paper deals with two new species belonging to *Lyophyllum* P. Karst. emend. Kühner s.l., *L. fuscobrunneum* and *L. impudicum*, collected in La Palma, Canary Islands, by Rose Marie Dähncke. Three papers concerning *Lyophyllum* species from La Palma have been already published in the recent past by the present authors (Dähncke et al. 2009, 2010; Vizzini & Contu 2010) but it is obvious that this genus is very well represented in this island and that careful studies could easily lead to the discovery of new taxa since nobody other than RMD has conducted extensive collections and studies on it there.

Materials & methods

The macromorphological descriptions follow the detailed field notes taken for each collection on fresh material. The micromorphological descriptions are based both upon study of fresh and herbarium material. Dried material was revived in 2% KOH and stained in Congo red and Phloxine B. Cotton Blue was used to highlight the siderophilous granulation in the basidia (Baroni 1981). Spore measurements are based on means of 30 spores. The width of basidia was measured at the thickest part, and the length was

measured from the apex (sterigmata excluded) to the basal septum. We followed the taxonomic concept of Bon (1999) and Kalamees (2004) for the *Lyophyllaceae* because a natural (molecular based) classification has not yet been proposed for this group, despite the important preliminary study by Hofstetter et al. (2002). Author citations follow the IPNI Authors website and the Index Fungorum Authors of Fungal Names website. Herbarium abbreviations are according to Thiers (2010). All examined material is housed at WU (Herbarium of the Department of Plant Systematics and Evolution, Faculty of Life Sciences, Universität Wien). Latin descriptions of the new taxa are deposited in MycoBank (http://www.mycobank.org).

Taxonomy

Lyophyllum fuscobrunneum Dähncke, Contu & Vizzini, **sp. nov.** FIGS. 1a, 2a–c

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Pileus 3–8 cm latus, parce carnosus, convexus, castaneo-brunneus, ad medium fusco-brunneus et obtuse lateque umbonatus, siccus, estriatus, hygrophanus, aetate pallescens. Lamellae latiusculae, subconfertae, uncinato-adnatae, cremeae deinde griseo-brunneae, nigrescentes. Stipes 4–6 × 1–1.5 cm, cylindricus, haud bulbosus, albus vel leviter brunneus, politus. Caro parce conspicua, alba, cartilaginea, nigrescens. Odor saporque farinae recentis. Sporae 5.2–6.7 × 3.7–4.5 µm, late ellipsoideae vel ellipsoideae, obtusae, leves. Basidia 30–50 × 7–9 µm, tetraspora. Cellulae marginales 20–35 × 3–7.5 µm, plerumque fusiformes vel cylindro-flexuosae. Pilei cutis ex hyphis iacentibus, radialibus vel laxe intertextis, 2–8 µm latis efformata. Fibulae numerosae.

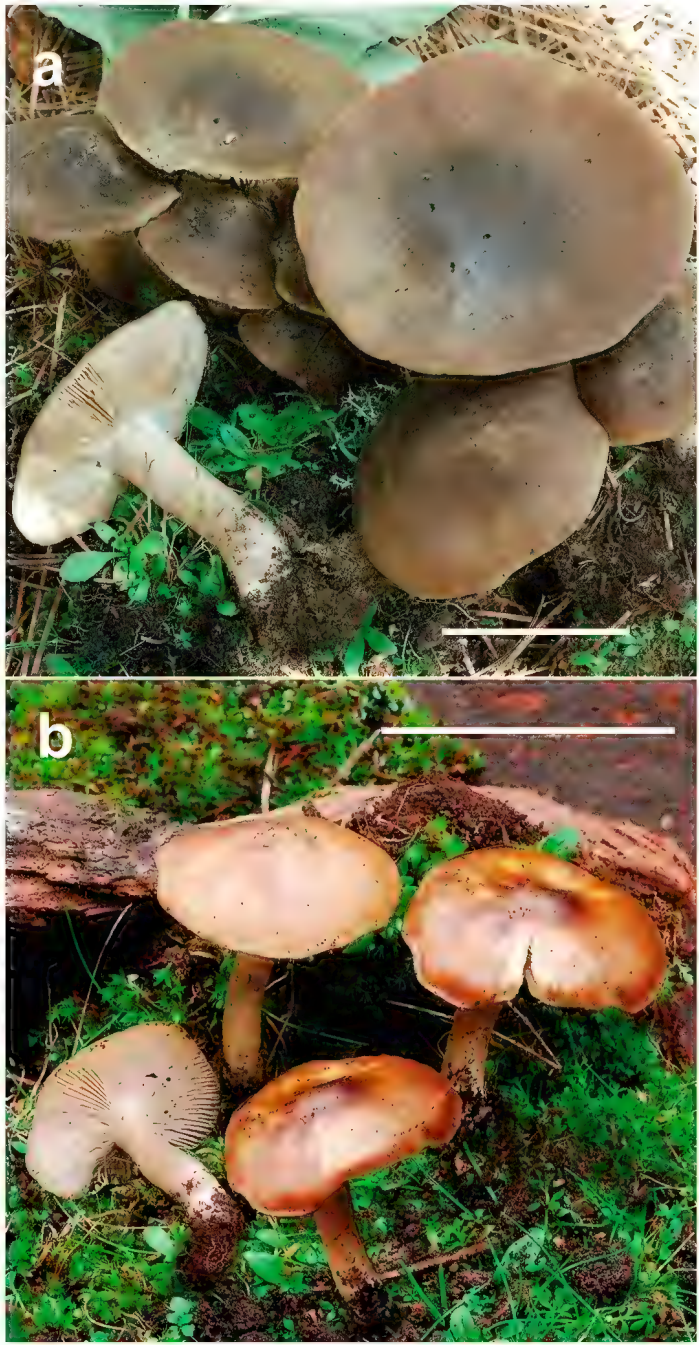
HOLOTYPUS: *Hispania, Insulae Canariae, in insula La Palma dicta, ad locum dictum Hoyo del Rehielo, 26.XI.2009, leg. R.M. Dähncke (WU 30641).*

ETYMOLOGY. The specific epithet, derived from the Latin adjectives *fuscus* (dark) and *brunneus* (brown), refers to the dark brown-pigmented pileus.

PILEUS 3–8 cm wide, not very fleshy, elastic-cartilaginous, at first broadly convex with an inrolled margin, then expanding, at centre with a broad, low and obtuse umbo, dry, glabrous, chestnut-brown with a dark brown to fuscous centre, hygrophanous and fading with age, non-striate, without pruina. LAMELLAE moderately close, uncinate-adnate, thin, cream then greyish-brown with age, staining brown then black when bruised. STIPE 4–6 × 1–1.5 cm, cylindric-equal, without an inflated basis and lacking a bulb, polished, white, stuffed with a thick white pith. CONTEXT thick in the disk but progressively thinning towards the pileus margin, white, staining black; smell and taste mealy. SPORE PRINT white.

SPORES 5.2–6.7 × 3.7–4.5 µm, on average 6.25 × 4.22 µm, hyaline, cyanophilous, carminophilous, broadly ellipsoid to ellipsoid, smooth, with a single, central, ellipsoid oil-drop, apex obtuse (FIG. 2a). BASIDIA 30–50 × 7–9 µm, clavate, somewhat hygrophoroid, four-spored, with basal clamp-connection (FIG. 2b); SUBHYMENIUM made up of elongate to inflated elements. HYMENOPHORAL

FIGURE 1 (right). Basidiomes. a. *Lyophyllum fuscobrunneum*. b. *L. impudicum*. Scale bars = 4 cm


a
b

TRAMA regular, consisting of hyaline hyphae often turning golden brown in 2% KOH. MARGINAL CELLS (cheilocystidia) 20–35 × 3–7.5 µm, mostly fusiform to cylindro-flexuose, inconspicuous, hyaline, thin-walled (FIG. 2c). PILEIPELLIS a cutis of differentiated, repent to loosely interwoven, cylindrical, smooth hyphae, 2–8 µm wide, with dominant parietal pigment; suprapellis an ixocutis of very thin elements 2–3 µm wide; subpellis and pilei trama with progressively wider hyphae. STIPITIPELLIS a cutis of elongate hyphae. CLAMP CONNECTIONS present at all septa. THROMBOPLEROUS HYPHAE not observed.

HABITAT. Gregarious near *Cistus symphytifolius* Lam. (*Cistaceae*) in a forest dominated by *Pinus canariensis* C. Sm. (*Pinaceae*). Autumn. Thus far known only from La Palma, in the Canary Islands.

COMMENTS. Among the closest European species, *Lyophyllum bonii* Contu (Consiglio & Contu 2002) has a paler pileus with no umbo, decurrent lamellae, and larger, less elongate spores. *L. ignobile* (P. Karst.) Clémençon shows a dark brown pigmented pileus, but differs in having different, non-mealy smell and taste, more elongate and narrower spores, definitely shorter and non-hygrophoroid basidia, and encrusted pigment in the pileipellis (Clémençon 1982, 1986; Bon 1999; Consiglio & Contu 2002). The recently described *L. brunneo-ochrascens* E. Ludw. has an umbilicate pileus and larger spores ("7–9.5 × 4.5–6 µm" in the type; Ludwig 2001) whilst *L. pulvis-horrei* E. Ludw. & Koeck (Ludwig 2001), also with small, broadly ellipsoid spores, differs in having adnate to decurrent gills, a different smell, and smaller basidia. Among the extra-European species keyed out by Clémençon & Smith (1983) *L. fistulosum* Clémençon & A.H. Sm. and *L. gracile* Clémençon & A.H. Sm. have bigger spores, often longer than 8 µm, shorter basidia ("27–33" µm long), and a more farinose-rancid smell. Finally, *L. chamaeleon* Clémençon & A.H. Sm. is close to *L. fuscobrunneum* in having very elongate, "37–45 × 5–7" µm basidia and a context with a mealy smell, but its spores are longer, narrower, and very elongate ("7.1–8.8 × 3.5–4.4" µm in the type).

Lyophyllum impudicum Dähncke, Contu & Vizzini, **sp. nov.** FIGS. 1b, 2d f

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Pileus 2 3.5 cm latus, parce carnosus, convexus, ad medium umbonatus, in juventute cremeo-ochraceus deinde brunneus, ad medium fuscus, siccus, estriatus, hygrophanus, aetate pallescens, sericeus. Lamellae subconfertae, adnatae, albae, immutabiles. Stipes 3 4 × 0.3 0.6 cm, cylindricus, haud bulbosus, albus vel leviter brunneus, politus. Caro parce conspicua, alba, immutabilis. Odor gravis, malus; sapor non notatus. Sporae 4.5 6.7 × 3.2 4.5 µm, late ellipsoideae vel ellipsoideae, obtusae, leves. Basidia 25 35 × 7 8.5 µm, tetraspora. Cellulae marginales nullae vel inconspicuae. Pilei cutis ex hyphis iacentibus, radialibus, 3 10 µm latis efformata. Fibulae numerosae.

HOLOTYPUS: *Hispania, Insulae Canariae, in insula La Palma dicta, ad locum dictum Cumbre Vieja, 12.XII.2009, leg. R.M. Dähncke (WU 30642).*

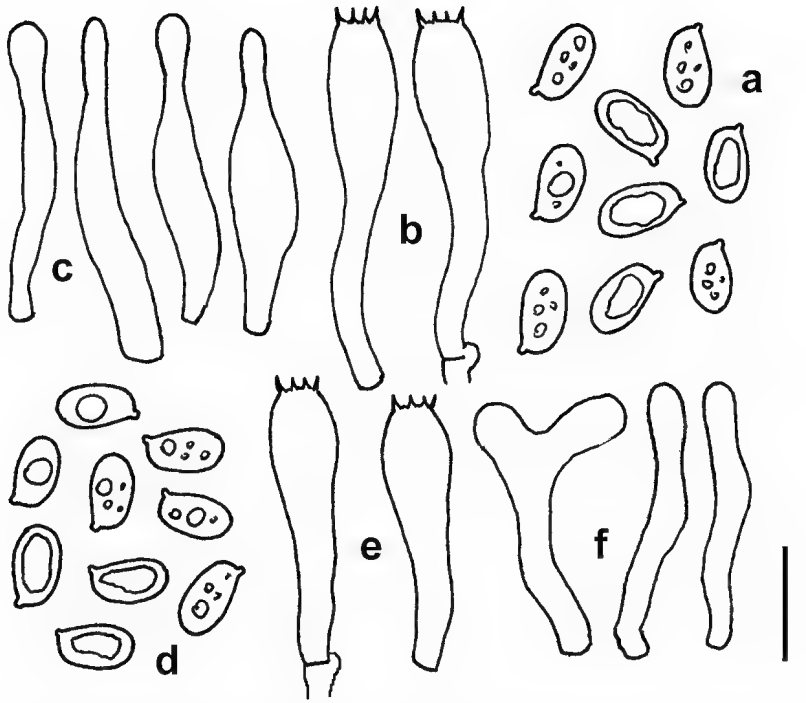

FIGURE 2. Basidiomes. Microscopical features. *Lyophyllum fuscobrunneum* (from the holotypus). a. Spores. b. Basidia. c. Marginal cells. *L. impudicum* (from the holotypus). d. Spores. e. Basidia. f. Marginal cells. Scale bar 10 µm

ETYMOLOGY. The specific epithet, derived from the Latin adjective *impudicus* (indecent, shameless, stinking, disgusting), means an evil-smelling, stinking species.

PILEUS 2–3.5 cm wide, not fleshy, not cartilaginous, at first broadly convex to convex-paraboloid, then convex but never expanding, with a small and rounded central umbo, dry, in young basidiomes pale ochre-cream then light brown with a darker, fuscous centre, hygrophanous and fading with age. LAMELLAE moderately close to close, adnate to adnate-emarginate, thin, white, not staining when bruised. STIPE 3–4 × 0.3–0.6 cm, subequal, white, brownish in old basidiomes, surface polished. CONTEXT thin, white, unchanging; smell unpleasant, disgusting; taste not recorded. SPORE PRINT white.

SPORES 4.5–6.7 × 3.2–4.5 µm, on average 6.15 × 4.27 µm, hyaline, cyanophilous, carminophilous, broadly ellipsoid to ellipsoid with an obtuse to slightly tapered apex, with a single, central, ellipsoid oil-drop, smooth, thin-walled, very slightly thick-walled only when old (FIG. 2d). BASIDIA 25–35 × 7–8.5 µm, clavate, four-spored, with basal clamp-connection (FIG. 2e); SUBHYMENIUM

made up of elongate to inflate, hyaline elements. HYMENOPHORAL TRAMA regular, composed of thin, hyaline hyphae. MARGINAL CELLS (cheilocystidia) inconspicuous, cylindric-flexuose to subfusiform (FIG. 2f). PILEIPELLIS a compact undifferentiated cutis of repent, cylindric, smooth hyphae, 3–10 µm wide, with dominant parietal pigment; subpellis scarcely differentiated; suprapellis not or very scarcely gelatinized. STIPITIPELLIS a cutis of elongate hyphae. CLAMP CONNECTIONS present at all septa. THROMBOPLEROUS HYPHAE not seen.

HABITAT. Under *Cistus symphytifolius*, in small groups. Autumn.

COMMENTS. *L. impudicum* is well circumscribed on the basis of the pale cream-ochraceous turning brown and umbonate pileus, the unchanging context with an unpleasant smell, and small, ellipsoid spores on four-spored basidia. There are no similar species in the literature since all the non-staining and non-caespitose species thus far known have globose to subglobose spores.

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Two new alectoronic acid-containing *Parmotrema* species from the coast of São Paulo State, southeastern Brazil

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ABSTRACT — Descriptions are presented for two new *Parmotrema* species containing alectoronic acid, *P. conidioarcuatum* and *P. pycnidiocarpum*, resulting from a survey of *Parmeliaceae* species in natural ecosystems and urbanized coastal areas of southeastern Brazil.

KEY WORDS *Parmotrema maraense, Parmotrema subrugatum*

Introduction

The genus *Parmotrema* A. Massal. is characterized by lobes with broad, rotund apices and naked lower margins, the absence of pseudocyphellae, the frequent occurrence of marginal cilia, simple rhizines, and thick-walled, ellipsoid ascospores (Brodo et al. 2001, Nash & Elix 2002). More than 300 species are known worldwide (Nash & Elix 2002), and about one third of them occur in Brazil.

Two new species containing alectoronic acid were discovered by the authors during research on the broad-lobed species of *Parmeliaceae* at the coast in São Paulo State, Brazil, at the same localities cited in Benatti et al. (2010).

Both new species lack vegetative propagules, and are corticolous in coastal mangrove or restinga forests. More morphological and chemical comparisons with other somewhat similar species can be found in Benatti (2005).

Material & methods

Specimens were distinguished by morphological characters using standard stereoscopic and light microscopes. Anatomical sections, including those of apothecia

and pycnidia, were made with a razor blade by hand. The chemical constituents were checked by spot tests with potassium hydroxide (K), sodium hypochlorite (C) and *para*-phenylenediamine (P), and also examined under UV light (360 nm). Chemical constituents were identified by thin-layer chromatography (TLC) using solvent C (Bungartz 2001), high performance liquid chromatography (HPLC) (Elix et al. 2003) and comparison with authentic samples.

We have encountered problems dealing with the many morphological terms present in the literature. For the purpose of this paper, we consider that lacinules represent adventitious, ribbon-like secondary outgrowths from the primary lobe margins (Marcelli et al. 2007). Lobules are similar, but short and rounded.

The diagnosis for each taxon refers exclusively to observations of the holotype specimen and the English descriptions and comments to all the material studied.

The species

Parmotrema conidioarcuatum Marcelli, Benatti & Elix, **sp. nov.** FIG. 1

MycoBank MB 518890

Species cum thallo similis Parmotrematis subrugati sed lobis latioribus, lacinulis multum longioribus et ramosioribus, lobulis aggregatis saepe submarginalibus formans, ciliis saepe majoribus, margine inferne eburnea solum subter apothecia vel lacinulis, apotheciis cum margine semper eciliata, sporis minoribus et conidia variabiliter arcuatis differt. atranorinam, chloroatranorinam, acidum alectoronicum, acidum β-alectoronicum, acidum dehydrocollatolicum et acidum dehydroalectoronicum continens.

HOLOTYPE: Brazil, São Paulo State, Municipality of Ilha Comprida, near the raft to Cananéia, low "restinga" vegetation, corticolous, under partial sunlight, leg. A.A. Spielmann, M.P. Marcelli, L.S. Canêz, & M.N. Benatti 964, 03-IV-2004 (SP).

ETYMOLOGY: This species is named after the arcuate conidia.

THALLUS up to 23 cm in diameter, subcoriaceous, ramulicolous, pale greenish gray but becoming dark gray in the herbarium, lobed to sublobed. LOBES irregularly branched, (3.5–)8.0–18.0 mm wide, crowded, weakly to strongly ascending, generally unattached; apices ± plane to subconvex, subrotund (primary lobes) to irregular and partially lacinulate; margin subcrenate to irregularly dissected, ±plane to ascending as it begins to form lacinules and/or apothecia, irregularly weakly incised, ciliate. UPPER SURFACE continuous to irregularly cracked, smooth to subrugose; MACULAE distinct and punctiform to linear when present on the stipes and amphithecia of the apothecia, laminal maculae rare and weak. LACINULES usually long, 0.3–17.5 × 0.2–2.3 mm, regularly spreading from lobe margins or apices, abundant at thallus center, initially simple but becoming irregularly branched, subcanaliculate, truncate or acute, ciliate, underside generally white, often mixed with tiny ciliate lobules which may develop into peculiar, scattered, submarginal, small, bouquet-shaped agglomerations, 3.0–8.0 mm wide. SOREDIA, PUSTULES and ISIDIA absent. CILIA black, simple or rarely furcate, 0.4–4.5 × ca. 0.05 mm, abundant at the lateral margins of the lobes but sparse at the apices. MEDULLA white, with scattered

FIGURE 1. The holotype of *P. conidioarcuatum* and the bouquet of lobules typical of the species (arrow and detail). Bar 1 cm.

orange, pigmented spots. LOWER SURFACE black, shiny, smooth to rugose or veined, free of rhizines when not contacting the substrate; MARGINAL ZONE shiny, usually brown, but turning white, cream or sometimes variegate under lacinules or apothecia, smooth to subrugose, 1.0–8.0 mm wide, naked; RHIZINES black, simple, furcate to irregularly branched, 0.10–2.30 (3.80) × 0.05 (–0.10) mm, frequent to abundant, in scattered groups. APOTHECIA submarginal or subapical, sometimes originating from subcanaliculate lobe apices, common, concave when young but becoming fissured and distorted with age, up to 25.2 mm wide, margins smooth to subcrenate, rarely lacinulate, always eciliate,

with very inflated stipes, amphithecia and stipe smooth at first then strongly veined and rugose or folded with age; disc brown, epruinose, imperforate; ASCOSPORES ellipsoid, 19.0–25.0 × 9.5–12.5 µm, epispore 1.5–2.0 µm thick; PYCNIDIA submarginal, common, particularly abundant on the lacinules, with black ostioles; CONIDIA variable inside a single pycnidium, bacilliform or short filiform, often arcuate, sigmoid or contorted into several different shapes, 5.0–7.5 × ca. 1.0 µm.

COLOR REACTIONS: upper cortex K+ yellow, UV–; medulla K+ weakly yellowish, C–, KC+ rose, P–, UV+ bluish green, and scattered spots of an orange, K+ dark reddish pigment.

TLC/HPLC: cortical atranorin (minor) and chloroatranorin (minor); medullary alectoronic acid (major), β-alectoronic acid (minor), dehydrocollatolic acid (minor), and dehydroalectoronic acid (trace).

COMMENTS: This species produces no vegetative propagules, has densely ciliate margins with abundant, long and branched lacinules, a brown lower marginal zone that turns white or cream colored only under lobes with apothecia and/or lacinules. The apothecia have smooth to subcrenate, ciliate margins, with ascospores up to 25.0 µm long, and a white medulla containing frequent spots of an orange, K+ dark reddish pigment.

However, the most striking characteristics of *P. conidioarcuatum* are the formation of tiny ciliate lobules, especially at submarginal parts of the thallus, lobules which eventually aggregate into small bouquets, and, most notably, the bacilliform or short filiform conidia that are often variably arcuate (arched, sigmoid, sinuous, subcrescent, somewhat unciform and several other shapes) intermixed with some linear conidia. Together, these characters readily distinguish *P. conidioarcuatum* from other *Parmotrema* species containing alectoronic acid. It is also one of the species with the broadest lobes in the genus.

We found no reference to *Parmotrema* species bearing similar small bouquets of lobules, nor are they present in any other Brazilian species known to us. Initially, we considered the possibility that this was due to some kind of deformation of the lacinules, but these lobules are clearly very common and consistent, differing in shape from the lacinules and with such regularity on the upper cortex, that they constitute a very unique morphological characteristic of the species. In addition, we had access to other specimens collected from inland São Paulo State, which have the same lobular bouquets (in early development), arcuate conidia and the other specific characteristics.

At first glance, *P. conidioarcuatum* might be mistaken for *P. subrugatum* (Kremp.) Hale, which invariably has unciform conidia (4.0–6.0 µm) and larger ascospores (26.0–39.0 µm). Sterile specimens of *P. subrugatum* (M!) are readily distinguished by the much narrower, more sparingly ciliate lobes,

the continuously white borders under the margins of all lobes, the absence of the lobular bouquets, and the simple to dichotomously branched lacinules (Benatti et al. 2010). In contrast, the lacinules of *P. conidioarcuatum* are more canaliculate, irregularly branched, densely ciliate, and grow crowded along the lobe margins; in addition they are sometimes more rugose due the accumulation of pycnidia.

Because of the dense marginal lacinulae and cilia, *P. conidioarcuatum* is probably more closely related to the *P. wainioi* subgroup, as they have similarly sized ascospores and conidia as well as a brown marginal zone on the underside. The unusually shaped conidia present in *P. conidioarcuatum* are believed to be derived from bacilliform or short filiform conidia.

Parmotrema maraense Hale (Hale 1990) has narrower lobes, with margins bearing sparingly branched lacinules, as well as more lacinulate and ciliate apothecia (Benatti 2005, Benatti et al. 2010), and short bacilliform conidia (Klaus Kalb, pers. comm.).

Parmotrema pycnidiocarpum Benatti, Marcelli & Elix, **sp. nov.** FIG. 2

MycoBank MB 518892

Species cum thallo simili Parmotrematis subrugati sed pycnidiis absentibus in lobis, apotheciis bullatis et multum pycnidiatis, cum pigmento aurantiaco disposito sub hymeniis et ad marginem differt. Atranorinam, chloroatranorinam, acidum alectoronicum, acidum α-collatolicum, acidum β-alectoronicum, acidum β-collatolicum, acidum dehydrocollatolicum, methyl pseudoalectoronatum, et methyl pseudo-α-collatolatum continens.

HOLOTYPE: Brazil, São Paulo State, Municipality of Iguape, Barra do Ribeira, between Suamirim "River" and the ocean, low "restinga" vegetation growing on inundated soil, on small tree branch in the wood, leg. M.P. Marcelli & O. Yano 6663, 17-VII-1989 (SP).

ETYMOLOGY: This species is named after the peculiar distribution of the pycnidia.

THALLUS up to 14.5 cm wide, submembranaceous, ramulicolous, pale grayish green, becoming darker in the herbarium, lobate to sublobate. LOBES 1.5–3.5(–5.0) mm wide, irregularly branched, imbricate to crowded, adnate, ascending when fertile, adnate to loosely adnate or unattached; apices ±plane to subconvex when fertile, subrotund to irregular; margin smooth near the apices, ±flat to ascending and subundulate, entire to irregularly incised, irregularly sublacinulate, ciliate. UPPER SURFACE continuous but becoming irregularly cracked with age, smooth to subrugose. MACULAE present only on some amphithecia and stipes, weak, punctiform, sometimes aggregated and linear. ADVENTITIOUS LACINULES sparse, simple or irregularly ramified, flat, 0.3–1.3 × 0.2–1.1 mm, irregularly distributed along the lobe margins, truncate or acute, underside concolorous with the lower margin or cream on fertile lobes, occasionally intermixed with some small irregular lobules. CILIA black, simple to rarely furcate or irregular, 0.1–1.5 × ca. 0.05 mm, frequent along the

margins but sparse at the lobe apices. MEDULLA white, invariably with orange pigmentation below the hymenium as apothecia mature, pigmented also at some apices and margins of older lobes. SOREDIA, PUSTULES and ISIDIA absent. LOWER SURFACE black, shiny, smooth to subrugose or weakly veined; MARGINAL ZONE shiny, brown, smooth to weakly veined, 0.5–3.5 mm wide, naked, turning cream under lobes with apothecia; RHIZINES black, simple, furcate or irregularly branched, 0.10–0.80(–1.30) × ca. 0.05(–0.10) mm, frequent to abundant, occasionally becoming agglutinated, usually scattered but partly in groups. Apothecia submarginal or subterminal, in part originating from subcanaliculate lobe apices, concave to urceolate, becoming rugose and distorted with age, up to 5.2 mm wide, eciliate, stipes highly inflated and bullate, margins smooth when young, then crenate, amphithecia and stipes smooth when young but becoming rugose or vertically folded; disc brown, epruinose, imperforate; ascospores absent, no asci observed in hymenia; pycnidia frequent to very abundant on the stipes and amphithecia, rarely submarginal on apotheciate lobes, with black ostioles; conidia short, unciform, 3.0–5.0 × ca. 1.0 µm.

COLOR REACTIONS: upper cortex K+ yellow, UV–; medulla K–, C–, KC+ rose, P–, UV+ bluish green, with a K+ dark reddish, orange pigment (unknown anthraquinone) below the hymenia of all mature apothecia.

TLC/HPLC: cortical atranorin (minor) and chloroatranorin (minor); medullary alectoronic acid (major), α-collatolic acid (minor), β-alectoronic acid (trace), β-collatolicacid(trace),dehydrocollatolicacid(trace),methylpseudoalectoronate (trace) and methyl pseudo-α-collatolate (trace).

COMMENTS: This alectoronic acid-containing species is characterized by narrow, ciliate lobes, the absence of vegetative propagules, eciliate apothecia with highly inflated, bullate stipes, pycnidia mainly on the amphithecia and stipes of the apothecia, very small unciform conidia, and an orange-pigmented medulla around the hymenium.

The orange medullary pigment (K+ dark reddish, possibly an anthraquinone) which is frequently deposited under and around the hymenia of apothecia, appears also in the medulla of some of the older lobes (often those beginning to form apothecia).

Particularly notable is the uncommon distribution pattern of the pycnidia. They grow almost exclusively on the amphithecia and stipes of the apothecia. Only rarely some submarginal pycnidia are found on the lobes, precisely where apothecial initials are being formed, around the edge of the juvenile disc.

The shape of the highly inflated stipes somewhat resemble large "skirts" as they begin to form, with the resulting lobe apices becoming more convex, especially the subapical ones. In this aspect, they differ from all other Brazilian species with inflated apothecia (Hale 1965, Fleig 1997, Louwhoff & Elix 1999,

FIGURE 2. Both sides of the holotype of *P. pycnidiocarpum*. Bar = 1 cm.

Eliasaro 2001, Kurokawa & Lai 2001, Canêz 2005, Spielmann 2005), where the apothecial pedicel is always ±cylindrical.

The conidia shape and size place this species in the *P. subrugatum* subgroup (Benatti et al. 2010). However, the lower marginal zone of *P. pycnidiocarpum* varies from brown on the distal lobes to cream on those bearing apothecia, in contrast to the almost entirely white margins of *P. subrugatum* (Benatti et al. 2010).

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New or noteworthy records of *Caloplaca* (*Teloschistaceae*) from Poland

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ABSTRACT *Caloplaca atroalba*, *C. dichroa*, *C. marmorata*, *C. oasis*, and *C. pseudofulgensia* are reported as new to Poland. Two other taxa: *C. albopruinosa* and *C. pusilla* are discussed in view of modern approaches. The species reported here were mainly collected in the Polish Carpathians. Additionally, *C. atroalba* is reported as new to Ukraine. Detailed taxonomic descriptions and comments are provided for the treated taxa.

KEY WORDS Central Europe, geographical distribution, lichenized fungi, taxonomy

Introduction

According to the checklist of the Polish lichen biota (Fałtynowicz 2003) and the most recent publications (Bielczyk 2003, Ceynowa-Giełdon & Adamska 2005, Wilk & Flakus 2006, Śliwa & Wilk 2008), 67 species of *Caloplaca* are known from Poland. The genus is relatively well-known in Poland, as it is in neighboring countries (Germany, Czech Republic, Slovakia, Ukraine). Among the authors that mostly contributed to this knowledge in Poland, Tobolewski (1956, 1958), Glanc & Tobolewski (1960), Nowak (1960, 1961), Nowak & Tobolewski (1975), and Alstrup & Olech (1988, 1992) are the best representatives.

The author has already reported on her 2004–2008 study of the taxonomic diversity of calcicolous *Caloplaca* species in the Polish Western Carpathians (Wilk 2008). In the course of this survey, several species were recognised as new to Poland. Some of them were reported recently (i.e., Wilk & Flakus 2006, Śliwa & Wilk 2008), and the remainder is presented here: *C. atroalba*, *C. dichroa*, *C. marmorata*, *C. oasis*, and *C. pseudofulgensia*. In addition, several noteworthy species were found in the area; two of them (*C. albopruinosa* and *C. pusilla*) are discussed in detail also in this study. Recently, the taxonomic placement

of the latter two species has been changed and they are presented here in their modern sense.

Material & methods

Specimens collected by the author and further material obtained from KRAM, KTC, UGDA, and herbaria of the Gorce National Park (GPN) and the Pieniny National Park (PPN) were examined. Additional reference material was studied in the Minnesota herbarium (MIN).

Morphological characters were measured on dry material using a dissecting microscope. Thallus color was compared with the color standard by Séguy (1936). Anatomical characters were measured from hand-cut sections and squash preparations mounted in water. The structure of paraphyses and cortical tissues were observed in 25% KOH (K). The granulation of tissues was observed in polarized light; pol +/ pol– indicates that granules do/do not reflect polarized light. Solubility of granules and/or crystals was tested with K and 65% nitric acid (N). Chemicals used in spot test reactions were: K, Lugol's iodine [0.3% IKI] (I) and N. The terminology for tissues follows Bungartz (2002), Ryan et al. (2002) and Gaya (2009). Systematic nomenclature of lichen associations and species concepts follow Roux et al. (2009), and additional lichen association data are provided by Gaya (2009).

Taxonomy

Caloplaca albopruinosa (Arnold) H. Olivier, Mém. Soc. Sci. Nat. Math. Cherbourg 37: 147 (1909).

Thallus endolithic or partially epilithic, thin, continuous to slightly cracked, white or grey (Séguy 1936, color code: 494), rarely limited by a dark grey prothallus; without vegetative propagules. Apothecia zeorine, abundant, in groups or more rarely slightly scattered, ±immersed at first, becoming sessile, round or slightly angular by mutual compression or flexuose, 0.2–1.2 mm diam., leaving pits in the rock when detached; disc at first slightly concave, then flat, black (Séguy 1936, color codes: 527, 528), more or less white or bluish due to pruina; proper margin persistent, thick, smooth (margin sometimes discontinuous and slightly cracked in young apothecia), distinctly raised above the disc, black, white or bluish due to pruina; thalline margin inconspicuous or suppressed. Parathecium well developed, thick, 65–170 µm, prosoplectenchymatous, lumina of cells elongated or slightly oval, outer part blackish. Amphithecium much reduced with few algae, cortex sometimes well developed up to 50 µm, paraplectenchymatous, cells with big round lumina, almost completely filled by small, yellow brown crystals (pol+, insoluble in K, soluble in N). Epihymenium granular, grey. Hymenium hyaline, without crystals, 80–115 µm tall. Paraphyses simple, constricted at septa, 1–2(–3) apical cells slightly thickened, up to 5 µm wide. Hypothecium hyaline, with many

oil droplets, paraplectenchymatous. Asci 8-spored, spores polaribilocular, thin-walled, 12–16.5 × 6.5–10 µm, isthmus 2–4(–5) µm wide. PYCNIDIA rather common, immersed in rock or thallus; ostiolum black, distinct; conidia colorless, ellipsoid to ovoid, 2–3.5 × 1–1.5 µm.

SPOT TEST REACTIONS: thallus K–, epihymenium K+ violet, KN+ violet-brown and outer part of parathecium K+ violet.

DISTRIBUTION AND ECOLOGY — *Caloplaca albopruinosa* has a Central Europe–Mediterranean distribution (Nimis et al. 1987). It grows on limestone and dolomite rocks in sun-exposed places in the subalpine and alpine belts (e.g., Alps, Apennines) and occasionally at lower elevations (Nimis 1993, Nimis et al. 1996a, Muggia et al. 2008). The species is characteristic of the association *Baglietoetum marmoreae* Roux 1978.

In Poland *C. albopruinosa* is rare, occurring in the Carpathians only in Tatra Mts. Eitner (1901) also reported the species from the Sudety Mts. It grows on limestone boulders in sun-exposed situations ca. 1200 m in montane glades.

EXSICCATES SEEN Arnold, Lich. Exsicc. 1222 (sub *Pyrenodesmia agardhiana*) (KRAM).

SPECIMENS EXAMINED **POLAND. Western Carpathians,** West Tatra Mts: Dolina Chochołowska valley, Polana Dudowa glade, alt. 1185 m, 49°14'58"N/19°49'37"E, 16 Jul 2004, *L. Śliwa 3151, 3169, K. Wilk 2157* (KRAM), Dolina Kościeliska valley, Przysłop Miętusi glade, 49°15'48"N/19°53'22"E, 15 Jul 2004, *L. Śliwa 3026* (KRAM).

COMMENTS — *Caloplaca albopruinosa* is characterized by an endolithic thallus and black, white pruinose apothecia with thick proper margin. The amphithecium is reduced with few algae. The epihymenium is grey and reacts K+ violet.

Together with *C. alociza* (A. Massal.) Mig., *C. badioreagens* Tretiach & Muggia and *C. erodens* Tretiach et al., *C. albopruinosa* forms a group of species characterized by black apothecia and an endolithic thallus. *Caloplaca albopruinosa* (= *C. agardhiana* auct., see Muggia et al. 2008 for nomenclature) is a problematic species, considered by some authors as a synonym to *C. alociza*. The presence or absence of crystals in the hymenium was a primary key character used to distinguish both species (Clauzade & Roux 1985, Nimis 1992), but recent studies indicate that crystals may occur in *C. albopruinosa* as well as in *C. alociza* (Muggia et al. 2008). Current concepts separate *C. alociza* from *C. albopruinosa* by a thin evanescent apothecial margin and by more rarely white pruinose apothecia. A brownish grey epihymenium (K+ brownish red) and paraphyses neither apically thickened and nor constricted at the septa distinguish *C. badioreagens* from *C. albopruinosa* (Tretiach & Muggia 2006). In comparison to *C. albopruinosa*, *C. erodens* has smaller apothecia with distinct thin proper margins and an endolithic-epilithic thallus producing grey soredia. This species occurs mostly as a sterile form (Tretiach et al. 2003).

Caloplaca atroalba (Tuck.) Zahlbr., Cat. Lich. Univ. 7: 68 (1930). PLATE 1

THALLUS epilithic, thin, areolate or continuous, brownish grey or creamy brownish (Séguy 1936, color codes: 234, 235, 338, 339), epruinose, rarely limited by a grey prothallus; without vegetative propagules. Areoles irregular, flat, with uneven surface. Thallus cortex paraplectenchymatous, without crystals; algal layer continuous; medulla prosoplectenchymatous. APOTHECIA zeorine, abundant, in the centre of the thallus, crowded, sessile from the beginning, round or angular by mutual compression, small, up to 0.5 mm diam.; disc flat, dark brown (Séguy 1936, color code: 117), epruinose; proper margin persistent, thin, concolorous with disc, epruinose; thalline margin usually persistent or ±reduced, but visible at least at the base of apothecia. Parathecium well developed, 50–65 µm thick, paraplectenchymatous, lumina of cells oval or round, outer part brown and without crystals. Amphithecium present, algae abundant, cortex poorly developed. Epihymenium brownish grey or pale brown. Hymenium hyaline, sometimes with fine crystals (pol–, insoluble in K), 85–115 µm tall. Paraphyses simple or slightly branched, constricted at septa, 1–3 apical cells slightly thickened, up to 5 µm wide, or apical cells not thickened. Hypothecium hyaline, sometimes with fine crystals (pol–, insoluble in K). Asci 8-spored, spores polaribilocular or occasionally 1-locular, thin-walled, 11.5–15 × 6–9.5 µm, isthmus ca. 1.7 µm wide, often incomplete or poorly formed. PYCNIDIA inconspicuous, immersed in thallus; conidia not observed.

SPOT TEST REACTIONS: thallus K+ violet, epihymenium and outer part of parathecium K+ violet.

DISTRIBUTION AND ECOLOGY — *Caloplaca atroalba* is widely distributed in the temperate zone of North America (Wetmore 1994, 2007) and is reported from Austria (Hafellner & Türk 2001) and Sweden (Santesson et al. 2004) in Europe. It grows on calcareous and occasionally non-calcareous rocks such as limestone, dolomite, or sandstone (data from MIN herbarium labels, Johnsen 1965, Wetmore 1994, Hafellner 2001).

In the Carpathians (Pieniny and Tatra Mts) and Góry Świętokrzyskie Mts, the species grows on limestone and calcareous sandstones in sun-exposed sites up to 1100 m. This is the first report of the species from Poland and Ukraine.

SPECIMENS EXAMINED — **POLAND. Western Carpathians,** Pieniny Mts, limestone outcrops by Czorsztyn Castle, alt. ca. 600 m, 49°26'11"N/20°18'48"E, 5 Jun 2005, *K. Wilk 3470b* (KRAM); West Tatra Mts, Dolina Chochołowska valley, Polana Chochołowska glade, alt. 1105 m, 49°14'16"N/19°47'47"E, 16 Jul 2004, *L. Śliwa 3118* (KRAM). **Góry Świętokrzyskie Mts,** Kielce County: Wesoła town above Wierna Rzeka river, hill near railway, 31 Aug 1976, *K. Toborowicz* (KTC-6332).

ADDITIONAL MATERIAL EXAMINED — **UKRAINE. Khmelnitskyi region,** Kamianets Podilskyi district, National Park "Podilskyi Tovtry": Kitaihorod, 15 km SE of Kamianets Podilskyi, 48°38'25"N/26°46'58"E, 24 Jun 2003, *P. Czarnota 3793* (KRAM), Bagota on the Dniester near Stara Ushytsia, 30 km SE Kamianets Podilskyi, alt. 259 m, 48°35'10"N/26°59'57"E, 25 Jun 2003, *J. Kiszka* (KRAM-L 63304).

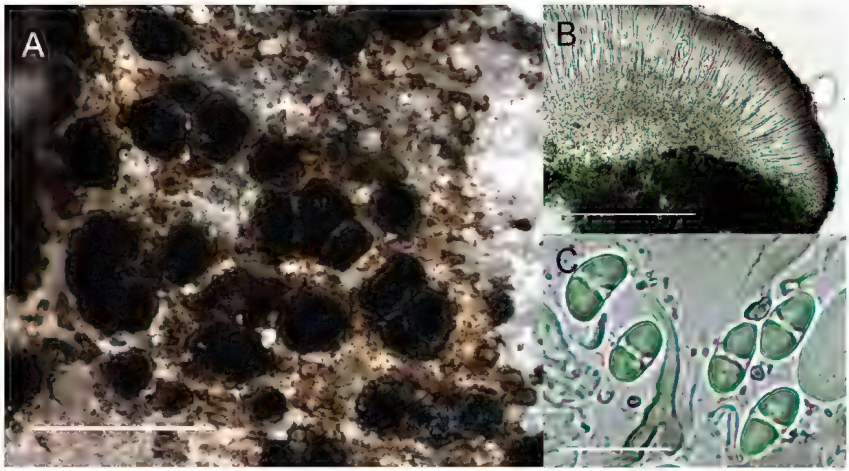

PLATE 1. *Caloplaca atroalba* (*L. Śliwa 3118*, KRAM): A – habit; scale bar = 1 mm. B – vertical section of apothecium; scale bar = 150 µm. C – ascospores; scale bar = 20 µm.

COMMENTS — *Caloplaca atroalba* is characterized by a creamy brown, epruinose, areolate thallus. The brown, epruinose apothecia usually have a persistent thalline margin. Spores have a thin isthmus (ca. 1.7 µm diam) and are often poorly formed. The epihymenium is brown and K+ violet.

Caloplaca atroalba can be similar to *C. albovariegata* (B. de Lesd.) Wetmore, *C. chalybaea* (Fr.) Müll. Arg., *C. diphyodes* (Nyl.) Jatta, *C. pratensis* Wetmore, and *C. variabilis* (Pers.) Müll. Arg. According to Wetmore (1994), *C. atroalba* is more common in Europe than currently thought but has been often misidentified as *C. diphyodes*. The latter, however, differs by producing spores with a wider isthmus (> 3 µm diam.) and a shorter (usually ≤ 100 µm) hymenium. An additional distinguishing feature is the parathecial structure, which is prosoplectenchymatous in *C. diphyodes* (Wunder 1974, Oksner 1993, Kondratyuk et al. 2004).

Caloplaca albovariegata can be distinguished from *C. atroalba* by its distinct grey areolate thallus with areoles constricted at the base. Moreover, *C. albovariegata* has a discontinuous algal layer (algal clusters separated from one another by vertical columns of hyphae) and a necral layer over the cortex. The species is known only from North America, but Wetmore (1994) notes that it probably also occurs in Europe.

Caloplaca atroalba may also be confused with *C. chalybaea* and *C. variabilis*, even though in the two latter species thalli and apothecia are covered by conspicuous white pruina (hyaline crystals visible in section, pol+, insoluble in K) and produce spores with considerably wider isthmuses. Moreover, the apothecia of *C. chalybaea* are ± immersed in the thallus and have a hypothecium

that consists of distinct rows of small isodiametric cells (Wetmore 1994). Finally, Wetmore (2009) recognises *C. atroalba* as a non-sorediate counterpart of *C. pratensis,* recently described from North America.

Caloplaca dichroa Arup, Lichenologist 38: 13 (2006).

THALLUS epilithic, 0.1–0.4 mm thick, granular-areolate, sometimes with small indistinct lobes at margin, yellow, yellowish orange or dark orange (Séguy 1936, color codes: 256, 257, 201), often occurring with yellow and orange thalli side by side, prothallus seldom present, concolorous with thallus. Areoles continuous to scattered, irregular, flat to convex, often with slightly incised margins; blastidia and granules numerous on the surface and margin of areoles, usually covering most of the thallus, 30–90 µm diam. Thallus cortex very thin, up to 10 µm; medulla with many yellow crystals. APOTHECIA zeorine, present or absent, not abundant, scattered in the centre and near margins of the thallus, slightly immersed at first, then sessile, round or flexuose when old, 0.5–1.5 mm diam.; disc flat, somewhat convex in old apothecia, orange (Séguy 1936, color codes: 211, 246); proper margin concolorous with disc or slightly paler, smooth; thalline margin thick and prominent at first, becoming reduced and visible at the base of apothecia, smooth or crenulate, or alternatively ±persistent and only slightly reduced. Parathecium thin or thick, prosoplectenchymatous, lumina of cells oval or round. Amphithecium often reduced and visible only at the base of apothecium, algae abundant and in groups. Epihymenium granular, brownish yellow. Hymenium hyaline, 65–95 µm tall. Paraphyses simple, slightly branched or anastomosing, with oil droplets, 1–3(–5) apical cells thickened, up to 5–8 µm wide, or apical cells not thickened. Hypothecium hyaline, with oil droplets, prosoplectenchymatous. Asci 8-spored, spores polaribilocular, thick-walled (1–2 µm wide), 10–15 × 5–8 µm, isthmus 2.0–4.5 µm wide. PYCNIDIA common, numerous, immersed; ostiolum orange; conidia colorless, bacilliform 2–3(–4) × 1 µm.

SPOT TEST REACTIONS: thallus K+ purple, medulla K–, epihymenium K+ purple.

DISTRIBUTION AND ECOLOGY — *Caloplaca dichroa* is widespread in Europe (Arup 2006, Vondrák et al. 2009), where it grows on calcareous substrata such as limestone rocks, boulders, pebbles, and (rarely) concrete and mortar (Arup 2006). It prefers sun-exposed sites, occurs at varying altitudes (Arup 2006, Vondrák et al. 2007), and is typically inland, away from maritime influences (Vondrák et al. 2009).

The species seems to be common in the Carpathian (Pieniny, Tatra, Beskid Mały) mountain ranges on limestone and calcareous sandstones mainly in sun-exposed sites, usually on south-facing vertical rock walls or upper rocky outcrops at 500–1800 m. This is the first report of the species from Poland.

Specimens examined — **Poland. Western Carpathians,** Beskid Mały Mts, Zamczysko above Łysina village, alt. 756 m, 49°44'52"N/19°18'17"E, 22 Sept 2005, *K. Wilk 3945b* (KRAM); Pieniny Mts, Zielone Skałki rocks by Czorsztyn lake, NE of Falsztyna, alt. ca. 580 m, 49°25'56"N/20°17'35"E, 6 Jun 2005 and 12 Nov 2005, *K. Wilk 3484, 3485, 4137, 4138* (KRAM); West Tatra Mts: Wąwóz Kraków canyon, alt. 1100 m, 15 Jul 2004, *K. Wilk 2141* (KRAM), below Mnichy Chochołowskie, E of Skorusi Żleb, alt. 1300 m, 49°14'29"N/19°47'43"E, 19 Jul 2004, *K. Wilk 2177* (KRAM), Mnichy Chochołowskie, S slope, alt. 1488 m, 49°14'40"N/19°47'42"E, 19 Jul 2004, *K. Wilk 2183* (KRAM), Rzędy below Ciemniak, alt. 1800 m, 49°13'75"N/19°53'80"E, 11 Jul 2004, *K. Wilk 2109b, 2111* (KRAM).

Additional material examined — **Ukraine. Kamelnitskyi region,** Kamianets Podilskyi district, National Park "Podilskyi Tovtry", Privorita near Makiv, 10 km N of Kamianets Podilskyi, alt. 223 m, 48°47'27"N/26°38'41"E, 25 Jun 2003, *U. Bielczyk* (KRAM-L 48993).

Comments — *Caloplaca dichroa* is characterized by a yellow or dark orange granular-areolate thallus (often both color morphs occur side by side). It produces vegetative propagules, such as blastidia and granules that usually cover the thallus entirely. The distinctly thickened spore walls are diagnostic.

This species is included within the *C. citrina* group (Arup 2006). Specimens of *C. dichroa* with thin, weakly developed thalli may be difficult to distinguish from *C. citrina* (Hoffm.) Th. Fr., which is differentiated by its distinctly sorediate, yellow-colored thalli and thin-walled spores. *Caloplaca arcis* (Poelt & Vězda) Arup is distinguished from yellow-colored forms of *C. dichroa* by its thicker thallus with distinct marginal lobes and thin-walled spores. According to Vondrák et al. (2009), *C. dichroa* can also be confused with *C. austrocitrina* Vondrák et al. and *C. limonia* Nimis & Poelt, but the latter two species produce thicker thalli. Additionally, *C. limonia* produces larger vegetative diaspores and a more intensely yellow thallus.

Caloplaca calcitrapa Nav.-Ros. et al., which also produces thick-walled, sand-glass spores, is distinguished by its complete absence of vegetative diaspores (Navarro-Rosinés et al. 2000).

Caloplaca dichroa has often been confused with *C. coronata* (Kremp. ex Körb.) J. Steiner, which produces a more distinctly isidiate thallus and smaller, thin-walled spores (see Arup 2006 for comments).

Caloplaca marmorata (Bagl.) Jatta, Sylloge Lich. Ital.: 251 (1900).

Thallus endolithic, without prothallus. Apothecia pseudolecanorine, abundant, scattered or in groups, immersed in rock at first, then sessile, round or slightly angular by compression, 0.2–0.7 mm diam.; disc flat or slightly convex, more rarely strongly convex, rust to brownish orange (Séguy 1936, color code: 171, 172), epruinose; proper margin persistent, raised, particularly in young apothecia rather thick, smooth, slightly paler than disc; thalline margin not visible. Parathecium well developed, thick, 85–170 µm, prosoplectenchymatous,

lumina of cells oval. Amphithecium much reduced, algae grouped at the base of apothecium. Epihymenium granular, reddish yellow. Hymenium hyaline, 85–110 µm tall. Paraphyses simple or slightly branched, with irregular lumina, 1–4 apical cells slightly thickened, up to 5 µm wide. Hypothecium hyaline, without crystals and oil droplets, prosoplectenchymatous. Asci 8-spored, spores polaribilocular, thin-walled, 12.5–17 × 5.5–8.5 µm, isthmus 1.5–3.5 µm wide. PYCNIDIA not observed.

SPOT TEST REACTIONS: epihymenium K+ purple.

DISTRIBUTION AND ECOLOGY — *Caloplaca marmorata* is widespread in Europe, the Near East and north Africa (Navarro-Rosinés & Hladun 1996) and is also reported from North America (Wetmore 2007). It is a pioneer taxon occupying hard calcareous pebbles and rocks, e.g. pure limestone, marble, gypsum or sandstones (Nimis & Poelt 1987, Nimis et al. 1996b, Navarro-Rosinés & Hladun 1996). It grows in sun-exposed and dry microhabitats at varying altitudes (Nimis 1993, Navarro-Rosinés & Hladun 1996). The species is characteristic of the association *Caloplacetum lacteae-marmoratae* Roux 2009.

In the Carpathians, the species was found only in Pieniny Mts, where it grows on sun-exposed limestone sites at 500–1000 m. This is the first report from Poland.

SPECIMENS EXAMINED — **POLAND. Western Carpathians,** Pieniny Mts, near summit of Trzy Korony, 4 May 1957, *J. Nowak* (KRAM-L 4401); Małe Pieniny Mts: Dolina Białej Wody valley, near Jaworki village, 8 Jun 1968, *J. Nowak* (KRAM-L 18696), near Jaworki village, Sołtysie Skałki rocks, alt. 580 m, 49°24'20"N/20°32'30"E, 3 Jun 2005, *K. Wilk 3395* (KRAM), Jaworki village, 1888, *W. Boberski* (KRAM-L 20914).

ADDITIONAL MATERIAL EXAMINED — **UKRAINE. Kamelnitskyi region,** Kamianets poilskyi district, National Park „Podolskyi Tovtry": Chotiri Kavalyeri near Vyerbka, 13 km N of Kamianets Podilskyi, alt. 289 m, 48°48'24"N/26°35'54"E, 25 Jun 2003, *J. Kiszka* (KRAM-L 49147), Kitaihorod, 15 km SE of Kamianets Podilskyi, alt. 141 m, 48°38'25"N/26°46'58"E, 24 Jun 2003, *M. Kukwa* (KRAM-L 48718), Tovtra Vyerbyetska Reserve, alt. 289 m, 48°48'24"N/26°35'54"E, 25 Jun 2003, *L. Śliwa 1964* (KRAM), ibid., *A. Zalewska* (KRAM L 49328).

COMMENTS — *Caloplaca marmorata* is characterized by an endolithic thallus and rusty or brownish orange, small apothecia with well developed proper margins. The parathecium is thick, and the amphithecium is reduced but with algae usually visible at the base of apothecium. The epihymenium is yellowish red and the spores have a thin (< 3 µm) isthmus.

The species belongs to the *C. lactea* group (Navarro-Rosinés & Hladun 1996), within which it could be confused with *C. lactea* (A. Massal.) Zahlbr., *C. lacteoides* Nav.-Ros. & Hladun, and *C. nashii* Nav.-Ros. et al. *Caloplaca lactea* has orange apothecia, persistent and thin proper margins, and broadly ellipsoid spores. *Caloplaca lacteoides* is differentiated by yellow, more rarely brownish orange apothecia, simple paraphyses with strongly thickened apical cells, an upper parathecium that is distinctly paraplectenchymatous, and very long

(≤ 25 µm) spores. *Caloplaca nashii* produces paler apothecia that are orange and never brownish or rusty, a thin parathecium, simple paraphyses with narrow or slightly thickened apical cells, and distinctly narrower spores (see also Navarro-Rosinés et al. 2001).

Caloplaca oasis (A. Massal.) Szatala, Magyar Bot. Lapok 31: 120 (1932).

Thallus strongly reduced and only some small granules or areoles visible at thallus margin, pale yellow (Séguy 1936, color code: 290), rounded in shape; without prothallus and vegetative propagules. Apothecia pseudolecanorine or zeorine, abundant, crowded, sessile, round or angular by compression, 0.1–0.3 mm diam.; disc flat or slightly convex, orange (Séguy 1936, color codes: 196, 211), epruinose; proper margin very thin, not prominent, slightly raised or level with disc, smooth, concolorous with or slightly paler than disc; thalline margin much reduced, only in young apothecia visible. Parathecium thin, ca. 60 µm, prosoplectenchymatous, hyphae radiating with oval to elongated cells. Amphithecium ±reduced, with numerous algae, cortex poorly developed. Epihymenium granular, yellow. Hymenium hyaline, 75–85 µm tall. Paraphyses simple or slightly branched above, 1–2 apical cells thickened, up to 7 µm wide. Hypothecium hyaline, without crystals or oil droplets, prosoplectenchymatous, hyphae irregular, ca. 40–50 µm high. Asci 8-spored, spores polaribilocular, thin-walled, 8.5–13.5 × (4.0–)4.8–6.8 µm, isthmus 3–5 µm wide. Pycnidia not observed.

Spot test reactions: thallus K+ purple, medulla K–, epihymenium K+ purple.

Distribution and ecology — *Caloplaca oasis,* which is widespread in Europe (Arup 2009), is also reported from Western Asia (John et al. 2004) and North Africa (Thor & Nascimbene 2010). The species grows mainly on pure limestone, concrete and mortar (Arup 2009), where it occurs as free living or a parasitic lichen growing on endolithic *Verrucaria* s. lat. The species is characteristic for the associations *Aspicilietum calcareae* Du Rietz 1925 emend. Roux 1978 and *Caloplacetum citrinae* Beschel ex Klement 1955.

The species was found in the Carpathians (Gorce Mts) at elevations up to 550 m and in the Pomeranian lowlands, where it grows on calcareous sandstones and concrete. This is the first report of the species from Poland.

Specimens examined **Poland. Western Carpathians,** Gorce Mts, Park Dworski in Poręba Wielka, alt. 550 m, 5 Oct 1993, *P. Czarnota* (GPN 49/94). **Pomeranian Voivodeship,** Wdzydze Landscape Park: Czarlina village, near bus stop, 13 Sept 2006, *K. Wilk 7271* (UGDA), southern part of Wdzydze Tucholskie village, 53°58'05"N/17°55'32"E, 13 Sept 2006, *E. Adamska, W. Gruszka & L. Śliwa 3668a* (UGDA).

Comments — *Caloplaca oasis* is characterized by a reduced thallus and small dark orange apothecia with thin proper margins.

Arup (2009), who discusses the species in detail in the recent monograph on the *C. holocarpa* group, notes that *C. oasis* has been often misidentified as *C. holocarpa* (Ach.) A.E. Wade, especially when growing on anthropogenic substrates. However, *C. holocarpa* produces more yellow-tinged apothecia with thicker, more prominent proper margins and spores with a broader isthmus (see Arup 2009). Well-developed parasitic *C. oasis* specimens may also be confused with *C. polycarpa* (A. Massal.) Zahlbr., which can be distinguished by larger apothecia with thicker margins and more distinct, thicker orange thalli (see Arup 2009 for more details).

Caloplaca pseudofulgensia Gaya & Nav.-Ros., Biblioth. Lichenol. 101: 69 (2009).

Thallus placodioid, rosettes 2–14 mm in diam., 0.1–0.4 mm thick, single or often forming bigger congregations, yellow, creamy yellow, orange, brownish orange, sometimes pale yellow to greenish yellow in shade (Séguy 1936, color codes: 246, 226–230, 211–215), thallus in the centre often whitish, surface rough, matt, pruinose (pruina concolorous with thallus); without prothallus and vegetative propagules. Marginal lobes 0.5–2 mm long and 0.1–0.5(–0.8) mm wide, closely adjacent to each other, richly and rather irregularly branched, convex in the centre of thallus, but flat and slightly broadening at tips; surface of lobes wrinkled and with delicate furrows along. Internal areoles flat to convex, irregular or resembling small sublobules; young thallus often lacking internal areoles, and then lobes forming from the centre. Thallus cortex distinct, thin or sometimes thick, paraplectenchymatous, without necral layer, with hyaline crystals (pol+, insoluble in K, soluble in N); algal layer ±continuous; medulla dense, with numerous hyaline crystals (pol+, insoluble in K, soluble in N). Apothecia zeorine or pseudolecanorine, abundant, in centre of thallus, sessile from the beginning, crowded, rarely scattered, round or angular and flexuose by compression, 0.2–0.8(–1) mm diam.; disc flat, then convex, dark orange or brownish orange (Séguy 1936, color codes: 186, 196, 201, 202), epruinose or slightly pruinose; proper margin persistent, slightly paler than disc; thalline margin present at first, then disappearing, thick, smooth or rough, even or slightly cracked, pruinose; sometimes thalline margin excluded from the beginning and only proper margin visible. Parathecium thin or thick, 25–60 µm, prosoplectenchymatous, lumina of cells oval. Amphithecium well developed or ±reduced, algae in groups or forming a continuous layer, sometimes amphithecial tissue loose and with holes, with a paraplectenchymatous cortex, with hyaline crystals (pol+, insoluble in K, soluble in N). Epihymenium granular, brownish yellow. Hymenium hyaline, 65–90 µm tall. Paraphyses simple or seldom slightly branched, 1–2(–3) apical cells thickened, up to 6–6.5(–7.5) µm wide. Hypothecium hyaline, with numerous oil droplets, prosoplectenchymatous. Asci 8-spored, spores polaribilocular, thin-walled, 7–13 × 3–6.5 µm, isthmus 2.5–5 µm wide. Pycnidia not observed.

Spot test reactions: thallus K+ purple, J–, N–, medulla K–, J–, N–, epihymenium K+ purple.

Distribution and ecology — *Caloplaca pseudofulgensia* is widespread in Europe (Gaya 2009), where it grows on calcareous rocks in warm and sun-exposed places on north-exposed vertical walls. This nitrophilous species belongs to the association *Caloplacetum arnoldii* Clauzade & Roux 1975 (corr. Roux 2009; see also Gaya 2009).

The species was found in the Carpathian Pieniny and Tatra mountain ranges, where it is not rare. It grows on limestone in sun-exposed to shaded sites, mostly on vertical rock walls at 500–1700 m. This is the first report of the species from Poland.

Exsiccates seen — Arnold, Lich. Exsicc. 1372 (sub *Physcia pusilla*) (KRAM).

Specimens examined — **Poland. Western Carpathians,** Małe Pieniny Mts, near Jaworki village, Sołtysie Skałki rocks, alt. 580 m, 49°24'20"N/20°32'30"E, 3 Jun 2005, *K. Wilk 3415* (KRAM); West Tatra Mts: Gładkie Jaworzyńskie, 18 Aug 1925, *J. Motyka* (KRAM-L 10527), Dolina Kościeliska valley, 16 Jul 1912, *W. Augustynowicz* (KRAM-L 571), Dolina Kościeliska valley, alt. 1000 m, 25 Jul 2004, *K. Wilk 2264* (KRAM), Stoły above Dolina Kościeliska valley, S slope, alt. 1400 m, 26 Jul 2004, *K. Wilk 2277* (KRAM), Wąwóz Kraków canyon, alt. 1100 m, 15 Jul 2004, *K. Wilk 2155* (KRAM), Wąwóz Kraków canyon, slope of Saturn, alt. 1500 m, 29 May 1959, *J. Nowak* (KRAM-L 8868), Kominy Tylkowe, by trail from Przełęcz Iwaniacka pass, alt. 1700 m, 5 Jul 1955, *J. Nowak* (KRAM-L 4796), Kalacka Turnia, above Suchy Żleb, 9 Jul 1957, *J. Nowak* (KRAM-L 2472), below Mnichy Chochołowskie, E of Skorusi Żleb, alt. 1300 m, 49°14'29"N/19°47'43"E, 19 Jul 2004, *K. Wilk 2178* (KRAM), W slope of Wielka Świstówka, in the direction to Kobylaszowy Żleb, 27 Jun 2004, *A. & M. Ronikier* (KRAM-L 55074); High Tatra Mts, Kalacka Turnia, alt. 1300 m, 9 Jul 1957, *J. Nowak* (KRAM-L 122).

Additional material examined — **Slovakia. Spiš,** Ľubovňa Castle, s.d., s.coll. (KRAM-L 20803).

Comments — *Caloplaca pseudofulgensia* is characterized by a placodioid, yellow to orange thallus covered by a distinct, concolorous pruina. Marginal lobes are flat and broad at tips and the lobe surfaces are wrinkled and furrowed. Dark orange apothecia contrast with the thallus.

The species belongs to the *C. saxicola* group (Gaya 2009), of which *C. pusilla* is the most similar. However, *C. pusilla* produces robust, convex, bigger, less branched lobes with surfaces that have neither wrinkles nor furrows and a thallus that is often salmon colored and white pruinose; furthermore its apothecia, which are initially immersed in the thallus, produce bigger spores.

Caloplaca saxicola (Hoffm.) Nordin differs from *C. pseudofulgensia* in its intensely orange, epruinose, smooth thallus. The thalline lobes, which are usually short to strongly reduced, convex, ascend slightly from the rock surface. Its apothecia, which are initially immersed in the thallus before becoming sessile and aggregated, produce bigger spores.

Morphologically, *C. pseudofulgensia* can be very similar to *C. aurea* (Schaer.) Zahlbr., a species that belongs to another taxonomical group but which produces 1- or polaribilocular spores with pointed apices and a greatly reduced isthmus and considerably longer (≤ 20 µm) size. *Caloplaca aurea* also produces a thicker, epruinose thallus that does not form the rosettes typical of *C. pseudofulgensia*.

Caloplaca pusilla (A. Massal.) Zahlbr., Annal. Naturhist. Hofmuseums Wien 4: 353 (1889).

Thallus placodioid, rosettes 2–10 mm in diam., 0.3–0.4 mm thick, single or usually forming bigger congregations, yellow ochre, pinkish or salmon, often whitish in the centre (Séguy 1936, color codes: 185, 189–190, 203, 205, 215), surface white pruinose, especially in the centre of thallus, where necrosis may also occur; without prothallus and vegetative propagules. Marginal lobes 0.5–2 mm long and 0.2–0.5 mm wide, closely adjacent to each other or sometimes overlapping, convex or flat and broad at tips. Internal areoles convex, rounded to elongated, or areoles absent and lobes forming from the centre. Thallus cortex thin to thick, 13–80 µm, scleroplectenchymatous, without necral layer; layer of algae not continuous; medulla loose, with or without crystals. Apothecia zeorine or pseudolecanorine, abundant, in centre of thallus, crowded or scattered, immersed at first, then sessile and often constricted at the base, rounded or angular by compression, 0.2–1 mm diam.; disc flat or slightly convex, dark orange, brownish orange or blood-red, more rarely reddish orange (Séguy 1936, color codes: 126, 161, 168, 171, 186, 202, 247), sometimes slightly white pruinose; proper margin persistent, slightly raised or level with disc, concolorous with disc or slightly paler; thalline margin persistent or disappearing, sometimes heavily white pruinose. Parathecium thin, 30–42 µm, sclero-prosoplectenchymatous. Amphithecium with numerous algae in groups or forming a continuous layer, sometimes amphithecial hyphae loose, cortex thick, with crystals. Epihymenium granular, brown or reddish golden. Hymenium hyaline, 60–90 µm tall. Paraphyses simple or slightly branched, 1–2(–4) apical cells thickened, up to 8(–10) µm wide. Hypothecium hyaline, without oil droplets and crystals, prosoplectenchymatouseous. Asci 8-spored, spores polaribilocular, thin-walled, 8.5–15 × 4–8.5 µm, isthmus 2.5–5 µm wide. Pycnidia infrequent, inconspicuous, immersed; conidia colorless, ellipsoid to bacilliform, 3 × 1.5 µm.

Spot test reactions: thallus K+ purple, J–, N–, medulla K–, J–, N–, epihymenium K+ purple.

Distribution and ecology — The widely distributed *Caloplaca pusilla* is probably cosmopolitan (Gaya 2009). The species grows on natural calcareous rocks and mortar, is known from varying altitude and exposures, and prefers eutrophic environments. It is characteristic of the association *Caloplacetum*

pusillae Du Rietz ex Kaiser 1926 but may also be found in the *Caloplacetum arnoldii* association (see Gaya 2009).

The species, which appears widely distributed in Poland, was found in the Carpathians (Gorce and Pieniny Mts) and in the lowlands in Warmian-Masurian province. More herbaria materials need revision to determine the true distribution, because *C. pusilla* has previously been filed under the name *C. saxicola* (Fałtynowicz 2003). In Poland the species occurs on limestone, calcareous sandstones, and (commonly) concrete in sunny sites at up to 980 m.

SPECIMENS EXAMINED **POLAND. Western Carpathians,** Gorce Mts, Boginka rock, above valley of Lubański stream, alt. 680 m, 15 Aug 1967, *K. Glanc* (KRAM-L 27476); Pieniny Mts: Popieska Skałka rock by Sronowickie lake, alt. 560 m, 49°24'48"N/20°19'50"E, 5 Jun 2005, *K. Wilk 3457* (KRAM), below summit of Trzy Korony, 4 May 1957, *J. Nowak* (KRAM-L 3786, KRAM-L 3790), Czorsztyn, s.d., s.coll. (KRAM-L 20787), Podłaźce, 10 Oct 1999, *J. Kozik* (PPN), Ociemny Wierch, 5 May 1955, *J. Nowak* (KRAM-L 3245), Zielone Skałki rocks by Czorsztyn lake, NE of Falsztyna, alt. 588 m, 49°25'56"N/20°17'35"E, Nov 2005, *K. Wilk 4131* (KRAM); Małe Pieniny Mts, Zabaniszcze, Góra Trzy Skałki Mt., alt. 740 m, Aug 1980, *K. Toborowicz* (KTC). **Warmian-Masurian province,** Giżycko County, Szymonka village, concrete bridge among meadows, 11 Jun 1974, *L. Olesiński* (KRAM-L 30631).

COMMENTS — *Caloplaca pusilla* is characterized by a placodioid, yellow ochre, pinkish or salmon colored thallus with long and ± convex marginal lobes that are tightly attached to the substratum.

Caloplaca pusilla, which was was treated as a synonym of *C. saxicola* for many years, has been just recently recovered as independent by Gaya (2009). *Caloplaca saxicola* differs from *C. pusilla* by an epruinose thallus with short to strongly reduced lobes that ascend distinctly from the rock, apothecia that are initially immersed in thallus and form finely, bigger and strongly aggregated clusters. The spores of *C. saxicola* are longer and narrower than in *C. pusilla.* To distinguish *C. pusilla* from *C. pseudofulgensia,* see remarks under that species.

Caloplaca pusilla often grows together with *C. arnoldii* (Wedd.) Zahlbr. and *C. clauzadeana* (Gaya) Nav.-Ros. & Cl. Roux. In comparison to *C. pusilla, C. arnoldii* has a distinctly reddish and smaller thallus, whereas *C. clauzadeana* has strongly convex lobes with yellowish tips and much narrower ellipsoid spores. For more details see Gaya (2009).

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Aspicilia subfarinosa, the correct name for *A. substerilis*

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ABSTRACT *Lecanora subfarinosa* is lectotypified and transferred to the genus *Aspicilia*, where it is recognized as the correct name for *A. substerilis*. *Lecanora farinosa* f. *subopegraphoides*, which is also lectotypified, is reduced to a synonym of *A. subfarinosa*. *Aspicilia subfarinosa* is newly reported from France, Israel, Iran, Iraq, and Turkey.

KEY WORDS *Ascomycetes*, Croatia, Greece, Italy, Syria

Introduction

We began our study of *Aspicilia* in SW Asia by examining all available types, which gave us an opportunity to look for earlier names for *A. substerilis* (Sipman 2007), a name recently published for a widespread, common species in our study area that has been confused with *A. farinosa* (Flörke) Hue. However, determination of the earliest available name was made difficult because so many new species have been described from the arid lands of SW Asia (Azerbaijan, Iran, Iraq, Lebanon, Syria and Turkey), SE Europe (Greece), and N Africa (Algeria, Egypt, Tunisia).

Aspicilia appears particularly diverse in SW Asia, where Julius Steiner described 44 representatives of *Lecanora* sect. *Aspicilia* (Steiner 1893, 1894, 1895, 1898, 1899, 1902, 1904, 1905, 1909, 1910, 1919a, b, 1921). R.G. Werner next described 18 new taxa in *Aspicilia* (Maire & Monod 1950, Werner 1936, 1938, 1954, 1956, 1957a,b, 1963, 1966, 1979). Ö. Szatala described 10 more species (Szatala 1929, 1931, 1939, 1941, 1943, 1956), and another 10 were proposed by A.N. Oxner (Kopaczevskaja et al. 1971, Oxner 1929, 1933, 1939, 1940). Altogether 55 (of 82) taxa described from SE Europe and SW Asia appeared as potential older names for *A. substerilis*. Of these, 27 taxa had either been originally proposed at or later raised to the species level prior to Sipman's

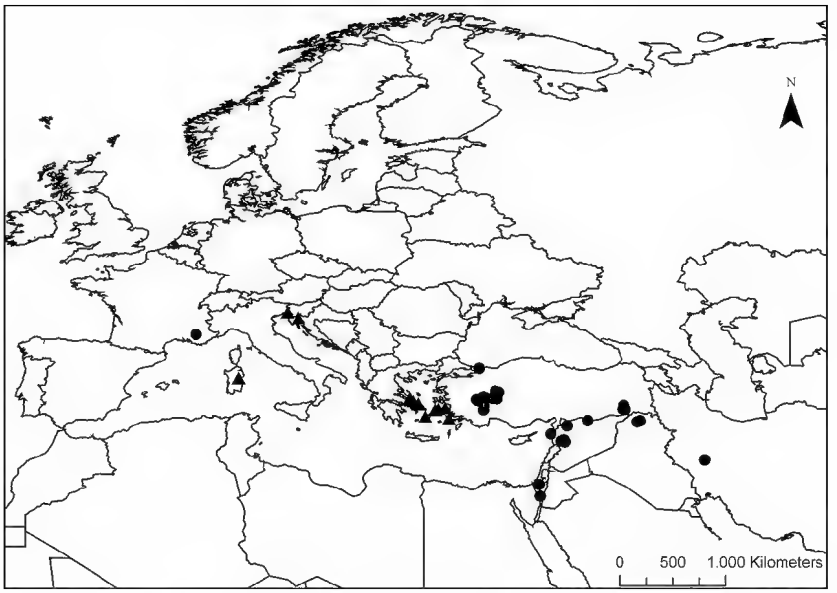

FIGURE 1. Distribution of *Aspicilia subfarinosa*.
▲ Localities cited in Sipman (2007), ● Specimens examined.

2007 description of *A. substerilis*. Examination of the 27 types revealed that one, *Lecanora subfarinosa*, was conspecific with *Aspicilia substerilis*. Among the remaining 28 infraspecific taxa, only the type specimen of *Lecanora farinosa* f. *subopegraphoides* appeared to be also conspecific with *Aspicilia substerilis*.

Materials & methods

The study presented here is based mainly on herbarium material kept in BC, BCN, BP, G, GZU, KW, W and WU. Most of type specimens in *Aspicilia* s.lat. described by A.N. Oxner, J. Müller Argoviensis, J. Steiner, Ö. Szatala and R.G. Werner have been revised. Extensive herbarium collections of *Aspicilia calcarea* and *A. farinosa* (incl. synonyms) were examined by the first author. The specimens were studied in the usual way by stereomicroscope and compound microscope, and all measurements (ascospores, conidia, and other apothecial structures) were made in microscopic preparations in tap water. To study ascus structure, Lugol's solution was added to sections and squash preparations after pre-treatment with 10% KOH.

Selected specimens were analysed for secondary metabolites by high performance thin-layer chromatography (HPTLC) in accordance with Arup et al. (1993), using a CAMAG Nanomat 4 sample applicator and a CAMAG Horizontal Development Chamber.

Additional specimen information obtained from collection labels is presented in English. Label data is abbreviated where necessary and reorganized for easy access with site information preceding collection data.

Taxonomy

Aspicilia subfarinosa (J. Steiner) Şenkard. & Sohrabi, **comb. nov.** FIG. 2

MYCOBANK MB 518413

≡ *Lecanora subfarinosa* J. Steiner, Ann. Naturhist. Mus. Wien 34: 38 (1921).

TYPUS: [Syria] Mesopotamia, in summo cacumine Tschil Miran montium Dschebel Sindschar, ad rupes substrato calcareo, ca. 1400 m, 9.6.1910, H.F. von Handel-Mazzetti 3297 (W 1920-827!-**lectotypus, hic designatus**, W 1929-2110!-isolectotypus)

Aspicilia substerilis Sipman, Biblioth. Lichenol. 96: 267 (2007), **syn. nov.**

TYPUS: "Greece, W Aegean, Nomos Evvias, Eparchia & Dimos Karistos: E Evvia, Agios Dimitrios Gorge, c. 350 m., calcareous, N-facing cliffs and boulders along dry stream in ravine, 30.9.2005, H. Sipman & Th. Raus 54305 [B-holotype, Sipman (2007)]

Lecanora farinosa f. *subopegraphoides* Werner, Bull. Soc. Bot. France 103(7–8): 462 (1956), **syn. nov.**

≡ *Aspicilia farinosa* f. *subopegraphoides* (Werner) S.Y. Kondr., in Kondratyuk & Zelenko, Ukr. Bot. Zhurn. 59: 603 (2002).

TYPUS: "Syrie: monts Ansarieh (Alaouites), région de Slenfé, versant Est dans le Cedretum, 1200–1300 m., sur les rochers calcaires, 27.8.1938, R.G. Werner" (BC!-**lectotypus, hic designatus**, BC!-isolectotypus).

ECOLOGY AND DISTRIBUTION— On the upper surface of well-lit, exposed limestone outcrops. Widespread in the Mediterranean (FIG. 1).

KEY CHARACTERS— This species is characterized by a thin, continuous to fissured, chalky-white thallus with farinose surface, apothecia separated from thallus by a fissure when dry, 4-spored asci, medium sized to large ascospores of 20–37 × 20–25 µm in size, rather short to medium sized conidia of 7–18 µm in length, and by the absence of secondary metabolites.

EXPANDED DESCRIPTION [based on Steiner (1921), Sipman (2007), and our own observations]— Thallus crustose, forming large patches usually over 5 cm wide, chalky white, farinose, continuous or more or less fissured around the ascocarps, 0.1–1.5 mm thick; margins without black prothallus, but with a groove between two adjacent thalli; cortex with paraplectenchymatous tissue constructed of less branched, moniliform-like and perpendicular hyphae and occasionally with a necrotic layer up to 60 µm thick; algal layer c. 60 µm thick, filled with small crystals; medulla white or occasionally becoming ochre in lower parts, composed of branched and reticulate hyphae and filled by larger, c. 5–10 µm wide crystals. Apothecia c. 0.2–0.8(–1.0) mm wide, immersed in the thallus and separated from it by a fissure when dry, without thalline margin, rounded or lobulate to elongate, often in groups seemingly originating from disintegrated larger apothecia, with grey, white-pruinose, concave disc and white, raised margins and sterile ridges (columellae); hymenium 100–150 µm tall, hyaline, clear, I+ persistently weak blue or partly turning to yellow; paraphyses in upper parts branched and submoniliform and 4–5 µm wide, in lower part 2.5–4 µm wide, loose, not branched; epithecium dark brown to

green, N+ green; hypothecium colourless, without algal layer below; asci of *Aspicilia*-type, subclavate, usually sterile, c. 95–130 × 23–44 µm; ascospores simple, hyaline, 2–4 in asci, c. 20–37 × 16–35 µm, usually absent. Pycnidia immersed, flask-like, with a black and punctiform ostiole; conidia filiform, straight, 6–18 × 0.6–1 µm.

SECONDARY METABOLITES— None detected by HPTLC.

ADDITIONAL SPECIMENS EXAMINED: **FRANCE: Forcalquier,** LES MOURES, Provence, 600 m — on calcareous stone, 18 Aug. 1969, *G. Clauzade & X. Llimona* (*BCN-lich. 41* as *A. farinosa*). **ISRAEL: C. Negev,** NEAR AVDATH — on calcareous stone, 16 Apr. 1974, *J. Garty* 36(7 as *L. farinosa*) (*GZU s.n* as *L. farinosa*); NEAR BEER-SHEBA, Nizana road — on hard limestone, 17 Mar. 1947, *Y. Gutter (BCN-lich. 279* as *L. farinosa)*. **IRAN: Lorestan Prov.,** ALIGUDARZ DISTRICT, Oshtran Kouh, 'Schuturun Kuh, Luristan' — no date, *S.D. Strauss* (*W s.n.* as *L. farinosa*); **East Azerbaijan**, MARAND DISTRICT 32 km N of Marand, 38.6763 N, 45.6573 E, 1440 m — 2 Nov. 2007, *M.Sohrabi, H.Sipman, U.Søchting & M.R.Asef.* (*hb. Sohrabi, MS010094*). **IRAQ: 'Mesopotamia',** SEIRAMUN prope Mossul, ca. 250 m — on calcareous stone, no date, *H.F. von Handel-Mazzetti,* as *L. farinosa* (*WU 40899*); **Kurdistania Turcica**, MOSUL, ON MÂR JAKUB — Jun 1910 *F. Nabelek* (*BP 42430* as *L. farinosa*); ca. 800 m — no date, *Nabelek* (*W s.n.* as *A. farinosa*). **SYRIA: Ain Arus,** between RAKKA & URFA, near Dschidle (Dicle), at Belich R. spring, ca. 350 m — on calcareous stone, 3 Jul. 1910, *H.F. von Handel-Mazzetti* 1843 (*W 1929-15582* as *L. farinosa*, with *L. platycarpa* f. *pruinosa* & *L. contorta* var. *glaucopruinosa*); **Dschebel Sindschar,** NEAR DSCHADDALE between Dschaddale & El Chattunije, ca. 600 m — on tufa-like calcareous stone, 10 Jun. 1910, *H.F. von Handel-Mazzetti 1543* (*WU 40901* as *L. farinosa*); EL MAGHARAD MT on SINDSCHAR, ca. 700–1000 m — on calcareous stone, 8 Jun. 1910, *H.F. von Handel-Mazzetti 1375* (*WU 40903* as *L. farinosa*); ca. 1000 m — on calcareous stone, 9 Jun. 1910, *H.F. von Handel-Mazzetti* 1437 (*WU 40897* as *L. farinosa*); TSCHIL MT. SUMMIT, ca. 1400 m — on calcareous stone, 9 Jun. 1910, *H.F. von Handel-Mazzetti 3295* (*WU 40894* as *L. farinosa*); **Haleb** (**Aleppo**), near train station, ca. 380 m — on calcareous stone, 15 Mar. 1910, *H.F. von Handel-Mazzetti 162* (*WU 40900* as *L. farinosa*); **Alaouites**, QASTAL EL MOAF, in Baër 40 km from Lattaquié (Muhāfazah) on the road to Antioche (Hatay) — on calcareous-siliceous stone, 3 Sep. 1938, *R.G. Werner*, (*BC s.n.* as *L. farinosa* f. *subopegraphoides* jointly with type specimens of *L. angulata*). **TURKEY: Bithynia**, OSMANKÖJ (OSMANKÖY), Gökszu (Göksu), in tree grove on limestone cliff, 300 m — 6 Oct. 1918, *I. Györffy de Szigeth* (*BP 42433* as "*L. calcarea* var. *concreta* f. *farinosa*"); BANK OF TIGRIDIS (TIGRIS) RIVER BETWEEN SERT (SIIRT) & DSCHESIRET-IBM-OMAR (CIZRE), near Fündük, ca. 1100 m — on calcareous stone, 18 Aug. 1910, *H.F. von Handel-Mazzetti 3036* (*W 1929-15666* & *WU 40893*, both as *L. farinosa*); between Balak & Fündük, ca. 500 m — on calcareous stone, 18 Aug. 1910, *H.F. von Handel-Mazzetti 3018* (*WU 40893* as *L. farinosa*) & *3041* (*WU 40904* as *L. farinosa*). **Afyon**, SÖĞÜT MTS, between Körpeli & Sarıköy, 3rd km, 37°54.96' N, 30°06.16' E, 1160 m — 24 Jun. 2009, *A. Şenkardeşler* (*EGE 39734*); AHIR MT, between Arızlar & Çepni, 3rd km, 38°34.02' N, 30°03.90' E, 1300 m — 23 Apr. 2010, *A. Şenkardeşler* (*EGE 39735*); between Yağcı & Çalhisar, 4rd km, 38°39.90' N, 30°04.26' E, 1490 m — 23 Apr. 2010, *A. Şenkardeşler* (*EGE 39736*); between Arharım & Karadirek, 2nd km, 38°36.00' N, 30°11.28' E, 1280 m — 23 Apr. 2010, *A. Şenkardeşler* (*EGE 39737*); between Gedikevi & Alibeyce, 1st km, 39°08.82' N, 31°04.56' E, 1130 m — 24 Mai 2010, *A. Şenkardeşler* (*EGE 39738*); between Salihler & Toklucak, 1st km, 39°02.88' N, 31°22.74' E, 980 m — 24 Mai 2010, *A. Şenkardeşler* (*EGE 39739*). SULTAN MTS, 0.5 km south of Kırca, 38°30.60'

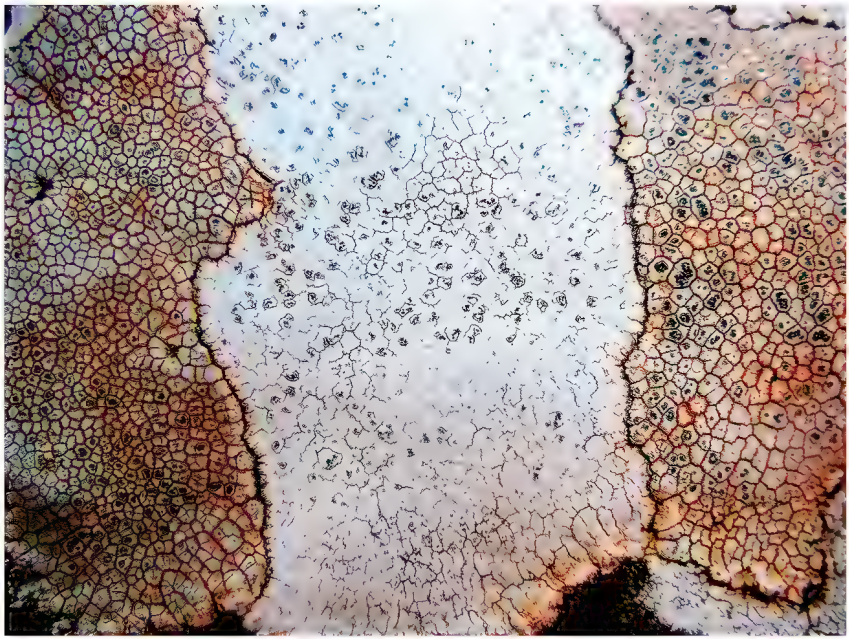

FIGURE 2. Comparison of the farinose, smooth surface with occasional very fine cracks of *Aspicilia subfarinosa* (EGE 39735, central), with the deeply cracked-areolate surface of *A. calcarea* (EGE 39735, left; EGE 39735, right).

N, 31°13.86' E, 1120 m 25 Mai 2010, *A. Şenkardeşler* (*EGE 39740*); between Pazarağaç & İnli, 5th km, 38°31.80' N, 30°49.38' E, 1060 m 25 Mai 2010, *A. Şenkardeşler* (*EGE 39741*). **Uşak**, BETWEEN DELIHIDIRLI & ÇOKAKLI, 0.5 km, 38°20.58' N, 29°34.56' E, 910 m 14 Mai 2010, *A. Şenkardeşler* (*EGE 39742*). ULUBEY CANYON, between Alfaklar & Paşalar, 4th km, 38°24.90' N, 29°30.42' E, 860 m 14 Mai 2010, *A. Şenkardeşler* (*EGE 39743*).

COMMENTS— Steiner (1915) provides a description with 4 ascospores per ascus for this species under the name *Lecanora farinosa* but admittedly never studied original material. Therefore he was unable to decide which of the two was correct, "*Lecanora farinosa* sensu J. Steiner" or "*A. farinosa* (Flörke) Hue", which has asci with up to 8 ascospores (Sipman 2007, Nordin & Roux 2009). All specimens of *Lecanora farinosa* sensu J. Steiner listed in Steiner (1910, 1915, 1921), Szatala (1927, 1941) and Şenkardeşler & Lõkös (2010) that we examined appear to belong to the species described by Sipman (2007) and not to "*A. farinosa* (Flörke) Hue".

The species *A. subfarinosa* was based on its longer conidia compared with *A. farinosa* sensu J. Steiner (11–18 µm vs. 7–12 µm). However, the cited specimen from Iran has conidia 11–14 µm long and a specimen from Israel, covered only with pycnidia, has longer (9–16 µm) conidia. Together with the similar external

thallus morphology for both species, the intermediate conidial lengths suggest that there are no informative morphological differences between *A. subfarinosa* and *A. farinosa* sensu J. Steiner.

The short description of *Lecanora farinosa* f. *subopegraphoides* is based only on the farinose surface and absence of areoles, which our study confirms. We also observed fissures around the ascocarps and 4-spored asci and detected no secondary metabolites. For these reasons, we synonymize here *A. farinosa* var. *subopegraphoides* and *A. substerilis* with *A. subfarinosa.*

The type specimen for a second form described by Szatala (1943), *A. farinosa* f. *ochracea*, has not been found even after diligent searches in BP, W, and WU. Probably it was lost during World War II. Verseghy (1964, 1968, 1974, 1981) did not report this type specimen from BP, either.

Aspicilia subfarinosa is very similar to and could be confused with *A. calcarea* (L.) Körb., but their surface structure is evidently different: *A. subfarinosa* has a farinose smooth surface with occasional very fine cracks, while *A. calcarea* has a deeply cracked-areolate surface (Fig. 2). Another difference between both species is the fissures around the apothecia in dry condition: *A. subfarinosa* has immersed apothecia, which are separated from the thallus by a fissure, while this is not a trait of *A. calcarea.*

Conclusion

Although *A. subfarinosa* and *A. farinosa* seem to be well defined species, the names have a complicated application history in some publications and checklists, chiefly in those dealing with southwest Asian and Mediterranean lichen flora. During this study it became clear that in the herbaria indicated above, a large number of examined specimens should be referred to *A. farinosa.* There are also some specimens in the *A. calcarea* complex, tentatively filed under *A. farinosa* or vice versa, deposited in different herbaria, that may need to be referred to *A. subfarinosa* after the number of ascospores per ascus and occurrence of fissures around the apothecia have been noted.

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Building the jigsaw puzzle of the critically endangered *Pleurotus nebrodensis*: historical collection sites and an emended description

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Abstract Historical collection sites of the critically endangered *Pleurotus nebrodensis* were retraced in Sicily (southern Italy) using recently rediscovered documents and through interviews with local people. These localities were visited in 2009 and checked for the presence of this rare mushroom, confirming a continuing decline in fruiting. An emended description of the taxon is provided based on observations on abundant recently collected material. The need for more incisive action to safeguard this valuable mushroom by the University of Palermo, Regional Park of Madonie Administration and policy makers is emphasized.

Key words — threatened macrofungi, mushroom collection, IUCN, *Cachrys ferulacea*, macromycete conservation

Introduction

After the death on 30 October 1887 of Giuseppe Inzenga, the mycologist who described *Pleurotus nebrodensis* [as *Agaricus nebrodensis*], Ferdinando Alfonso Spagna replaced him as director of the periodical Nuovi Annali di Agricoltura Siciliana and continued some of the mycological research started by Inzenga. In his paper on poisonous fungi growing in Sicily, Spagna (1902) also added a list of edible species appreciated by local populations for their organoleptic characters that included *P. nebrodensis*, "the most delicious mushroom growing in Sicily" (Inzenga 1863).

This mushroom, which has been a sought-after edible since ancient times, remains prized (Venturella 2005) today. The recent rediscovery of Spagna's paper

has permitted a new piece to be added to the ongoing "jigsaw puzzle" of the critically endangered *P. nebrodensis*. In addition to clarifying morphological, taxonomical, and genetic features and in situ and ex situ conservation strategies (Venturella 2000, Zervakis et al. 2001, Zervakis & Venturella 2002), we are now able to identify the historical collection sites of *P. nebrodensis* that Inzenga (1865-68) omitted from his publication, provide an emended description of the species, and evaluate its declining fruiting productivity over the last centuries.

Materials & methods

Based on data reported by Spagna over one hundred years ago, we conducted cartographic analysis, field investigations, and in-depth interviews of cattle farmers, working for many years in the Regional Park of Madonie (northern Sicily). Two localities were established and mapped (scale 1:50.000). Additional field investigations during spring 2009 verified the presence of basidiomata of *P. nebrodensis*. Macroscopic and microscopic features were evaluated according to Largent et al. (1977) and Largent (1986). Macroscopic features include pileus size, shape, and color; margin shape and surface; pileus surface and flesh; lamellar characters; stipe characteristics; presence or absence of veil on stipe; type of basidioma attachment; spore print. Colors refer to the RAL matching system. Microscopic features evaluated were hyphal system; hyphal wall; septations; hyphal branching; hyphal inflations; specialized hyphae; pigmentation; type of pellis; trama, position, type, shape, pigmentation and incrustations of cystidia; basidia and basidioles; spore features; chemical reactions. The collected basidiomata were identified, analysed in our laboratory, and are now preserved in the PAL fungal dried reference collection.

Specimens collected

Field investigations carried out in the Madonie area during spring 2009 confirmed the presence of a very low number of basidiomata of *P. nebrodensis* (Fig. 1). The detection of ripe and unripe basidiomata is complicated by excessive collecting by mushroom pickers. Progressive erosion of soils and changes in habitat at higher altitudes should also be considered as factors contributing to the decline of *P. nebrodensis*. In some years the irregular precipitation in the Madonie area heavily reduced fructification. Modern collections are limited strictly to localities belonging to the territories of Isnello and Petralia Sottana (only 50 kilometres apart) and Polizzi Generosa (a small town 16 km from Petralia Sottana). In the reported localities the number of ripe basidiomata observed from April to May usually did not exceed 25–30 in the more protected areas and 5–15 in habitats subject to a high anthropogenic pressure. The lowest collection locality is Mandria del Colle (1260 m) while the highest is Pizzo Carbonara (1979 m).

Original and emended descriptions

Pleurotus nebrodensis (Inzenga) Quél., Enchir. fung.: 148 (1886). Figs 1–2

≡ *Agaricus nebrodensis* Inzenga, Giorn. Reale Ist. Incoragg. Agric. Sicil. Palermo 1: 161 (1863). [Type (Venturella 2000): lectotype Inzenga (1863: fig. 1); epitype PC, *Agaricus nebrodensis*].

FIG. 1. *Pleurotus nebrodensis*. Basidiomata.

ETYMOLOGY: from the name of the Nebrodi mountain chain of northern Sicily. (Since old maps did not differentiate between the Nebrodi and Madonie mountain chains, botanists applied the name Nebrodi to both areas.)

ORIGINAL DESCRIPTION (Inzenga 1863): *Ag. magnus caespitosus, albus, vel dilute sub-flavus, pileo carnoso nargine revoluto, lamellis confertis lineari-lanceolatis, liberis, decurrentibus in stipite sublaterali, versus basim permixtis.*

Fungi umbilicum exprimentes, simul albi C.B.P. – Fungi plures simul, albi, ad arborum radices, esculenti J. B. Cup. H. Cath. pag. 80.

Pileus junior laevigatus, albus, subumbonatus, demum dilute flavus, irregulari, modo ex epidermide diffracia rimoso-tessulatus, gregarious, caespitosus, aliquando ob coacervata insitaque individua ramosus: 2 5 unc. latus, et ultra. Stipes rare centralis, supra dilatatus atque in pileo diffuses, brevis, subnullus, basi attenuates. Lamellae confertae tenues, lineari-lanceolate, longo decurrentes sub striarum forma versus stipitis basim productae. Lamellulae numerosae, breviores lanceolatae, longiores postice rotundatae. Caro fibrosa, subtenax, saporis gratissimi, ac odoris farina molitae, albida, sicca dilute-flava. Sporidia alba.

Agarico Eryngii DC. characteribus variis consimilis, sed magnitudine, colore albido pilei sporidiorumque, stipite breviore, lamellis confertis, angustis, lineari-lanceolatis omnino distinctus.

In montium culminibus Siciliae, Nebrodibus magis obvia e radicibus marcescentibus Elaeoselini Asclepii Bert., Opopanacis Chironii Koch., etc. Aprili, Majo nive dilabente. Esculentus!

EMENDED DESCRIPTION (based on recent collections): BASIDIOMATA sturdy and fleshy. PILEUS SIZE 3.0–14.5 cm (width), 3.7–13.5 cm (length); applanate, uplifted, shallowly depressed or convex in side view; ovoid or conchate in top view; unicolorous, light ivory, sometimes bicolorous, cream to ivory. MARGIN OF PILEUS plane, incurved, uplifted, incurved or involute in cross section, entire to eroded in surface view. SURFACE OF MARGIN entire or eroded, smooth. SURFACE OF PILEUS shiny or translucent, dry, cracking-glabre, smooth or glabrous. FLESH of pileus, cream, with consistency hard-tough to turgid, without color changes, sulphur-yellow when dry, 1–2 mm thick at the margin and 1–4 cm thick at the center. Taste mild and farinous. LAMELLAE 4–8 mm width, 2.5–7.5 cm length, attachment adnexed to decurrent, gills spacing subdistant to close, moderately broad in thickness, light ivory colored, margin of gills smooth to eroded, face of gills waxed, lamellulae present, extending one-half to one-third the length of gills. STIPE 1.4–3 cm width, 2.1–7.5 cm length, terete in cross section, equal to bulbose, slightly tapered to tapered at the base in longitudinal view. STIPE SURFACE smooth, light ivory colored. STIPE CONSISTENCY fibrous, flesh solid to stuffed. Stipe eccentrically or lateral attached to pileus, inserted in the root residues of *Cachrys ferulacea*, basal tomentum and veil absent. GROWTH HABIT solitary or connate. TYPE OF BASIDIOMATA ATTACHMENT: stipitate. SPORE PRINT light ivory to cream. SPORES 12.5–15.1(–18) × 5.2–6.1 µm, cream, heterotrophic, asymmetrical, phaseoliphorm, smooth, hyaline, guttulate. BASIDIA with basidioles, 4-spored, 40–50 × 10–11.5(–14)µm, sterigmata 3–4.5 µm. CHEILOCYSTIDIA 50–60 × 6.2–7.5(–9) µm, leptocystidia type, clavate, apex mucronate to capitulate. HYPHAL SYSTEM monomitic. HYPHAL WALL thin.

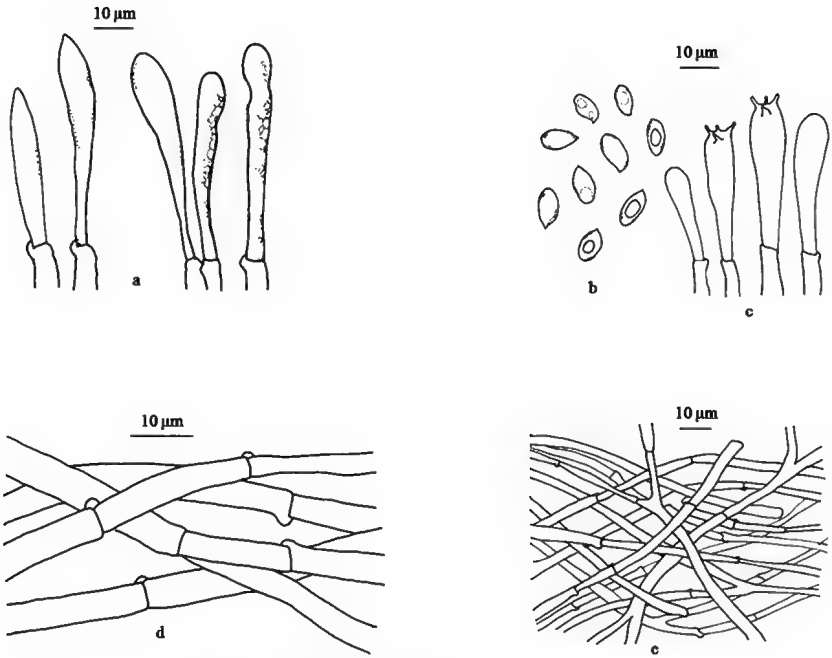

FIG. 2. *Pleurotus nebrodensis* microscopic characters:
a. cheilocystidia; b. basidiospores; c. basidia; d. hyphal system of hymenium; e. pellis.

HYPHAE septate with clamp connections. SPECIALIZED HYPHAE absent, no pigmentation. PELLIS topography in two layers, 5–10 µm width, enterocutis, element of pellis absent (FIG. 2).

SPECIMENS EXAMINED: All on *Cachrys ferulacea* (L.) Calest. (*Apiaceae*) root residues **ITALY. SICILY:** PROVINCE OF PALERMO, **Isnello**: Bevaio del Faggio, 1355 m, A. Saitta, 001, 5 May 2009 (PAL); Mandria del Colle, 1260 m, A. Saitta, 002, 24 April 2009 (PAL); Pizzo Carbonara, 1979 m, A. Saitta, 003, 28 May 2009 (PAL); Valle Pelata, 1745 m, A. Saitta, 004, 20 May 2009 (PAL); **Petralia Sottana**: Monte Ferro, 1906 m, A. Saitta, 005, 30 May 2009 (PAL); Monte Mufara, 1685 m, A. Saitta, 006, 26 May 2009 (PAL); Pizzo Carbonara, 1979 m, A. Saitta, 007, 28 May 2009 (PAL); Valle di Zottafonda, 1843 m, A. Saitta, 008, 29 May 2009 (PAL); Vallone Faguare, 1380 m, G. Venturella & ML Gargano, 009, 12 May 2009 (PAL); **Polizzi Generosa**: Cozzo Cerasa, 1687 m, A. Saitta, 010, 18 May 2009 (PAL); Monte dei Cervi, 1794 m, A. Saitta, 011, 23 May 2009 (PAL); Piano della Madonna, 1692 m, A. Saitta, 012, 3 May 2009 (PAL); Pizzo Colla, 1676 m, A. Saitta, 013, 8 May 2009 (PAL).

CULTURE PDA: pigments absent, reverse colour unchanged, dikaryotic colonies mostly presenting a loose submerged and suppressed aerial mycelium, more or less zonate and radial, growth margin even and regular, colour light ivory to ivory; thin-walled hyphae, hyaline in aqueous KOH and Melzer's reagent, with

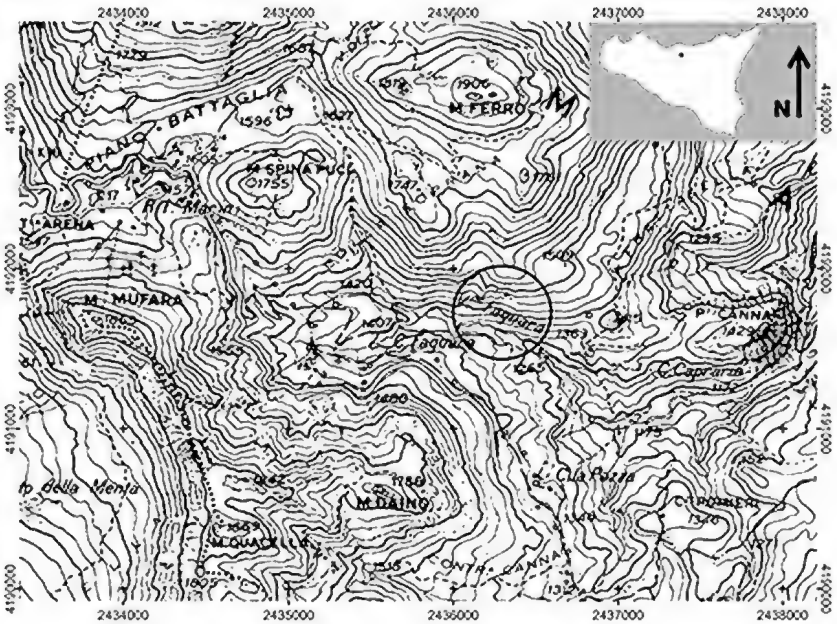

FIG. 3. Vallone Faguare locality (map scale 1:50000).

abundant clamp-connections, occasional production of microdroplets, singly on short secretory sterigmata on aerial hyphae (nematode trapping devices); optimal mycelium growth at 18–25°C (growth rate: 1.2 mm day^{-1}).

Discussion

Mr Norata, one of the cattle farmers interviewed, now a grey-headed gentleman in his 90s, provided the critical evidence enabling us to rediscover the historical collection sites of *P. nebrodensis*. In an enthusiastic and moving reconstruction of his working life in the pasture areas of Madonie, including memories of fear as a young boy during lonely nights guarding his cows, Mr Norata pointed out the sites which, at the beginning of the 20th century, were best for collecting this valuable mushroom. He also recounted his observation of the progressive decline of *P. nebrodensis*, which he attributed to intense exploitation of the area by cattle farmers and progressive loss of habitat due to road building. On the basis of Mr Norata's information and our field observations we identified the two collection localities reported by Alfonso Spagna, which he named as "Canna" and "Dragonara". These are fiefdoms of the property of Baron Nicolò Turrisi Colonna's heirs. The two localities, nesting in a deep valley of the Madonie Mountains (northern Sicily) named Vallone

Faguare (Fig. 3), currently belong to the lands of Petralia Sottana (province of Palermo). The precise coordinates (Gauss Boaga coordinate, ed. 1950) are 4191750 N and 2436300 E. Their vegetation is characterized by wide-ranging pastures of *Cachrys ferulacea* intensively exploited for grazing until 1989 when the Regional Park of Madonie was established.

The new rules introduced by the Madonie National Park Administration have not interrupted the stream of mushroom pickers stimulated by the high economic value of *P. nebrodensis* (50–60 euro per kg). After inclusion of this rare mushroom as Critically Endangered in the IUCN Red List of Threatened Species, G. Venturella (Head of the Laboratory of Mycology in Palermo) persuaded the Park of Madonie administration to issue a conduct code for mushroom pickers. Unfortunately, mainly due to a lack of trained staff, the level of practical control by forest rangers has been insufficient to prevent an increase in mushroom picker pressure on the collection sites. As a consequence, unripe basidiomata are collected every spring, increasing the decline of *P. nebrodensis*. Now, a very low number of ripe basidiomata is collected in the area of Vallone Faguare, and this present situation clearly contradicts Spagna's report in 1902 and the direct evidence gained through our interviews. At that time "abundant collections of the renowned mushroom" were reported by the local population. The presence of ripe basidiomata of *P. nebrodensis* has progressively decreased during the last centuries and the population is now severely fragmented (Venturella 2005). In 2009, an investigation carried out in Sicily (southern Italy) during the period of fructification (April–June) confirmed the negative trend and the need for more incisive action by the University, Park Administration, and policy makers to safeguard the existence of this important taxon.

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Diplotomma, Lecanora, and *Xanthoria* lichen species new to Turkey

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ABSTRACT — During a recent excursion in Kemaliye district of Erzincan region (Turkey), we sampled lichens from 6 localities, among which *Diplotomma hedinii, Lecanora valesiaca,* and *Xanthoria weberi* were determined as new to Turkey and the Middle East and *X. weberi* also new to Asia. Geographic distribution, substrate, chemistry, and comparisons with morphologically similar taxa are presented.

KEY WORDS — *Ascomycota*, biodiversity

Introduction

The lichen flora of Turkey, as in many parts of the world, is still incomplete. Although many recent studies (Candan & Turk 2008, Candan & Halici 2009, Halici & Güvenc 2008, Halici & Aksoy 2009, Yazici & Aslan 2009, Yazici et al. 2008, 2010a,b) contributed to the lichen flora of Turkey, unexplored regions of the country are still larger than the explored ones. Therefore, more studies are needed to form a complete lichen flora of Turkey.

Although part of Erzincan flora has been previously described (Yazici & Aslan 2003), no studies were carried out around Kemaliye district.

Material & methods

Some lichen species were collected from 6 localities in June and July 2009. Air-dried samples were examined with Nikon SMZ1500 stereomicroscope and a Nikon Eclipse 80i light microscope. Identifications were determined by consulting using books (Poelt 1974, Smith et al. 2009) and papers (Upreti & Chatterjee 1998, Aptroot et al. 2009, Lindblom 2006).

The Kemaliye district has many deep valleys, high mountains, and plateaus. Continental climate dominates the higher altitudes while a mediterranean-type

microclimate is present on the valley floors. The district's largest valley is Karasu Valley, and other valleys have been carved by branches of the Karasu River, which reaches Keban Dam Lake just beyond the district border. The presence of Keban Lake dramatically alters climatic conditions at the valley bottoms, where summer temperatures and humidity significantly increase compared to near regions (Bulut 2006). Dominant plant cover in the district is mountain steppe. Frequent patches of squat trees like *Quercus* sp. and *Juniperus* sp., as well as *Rosa canina* shrubs can be seen over on the hills. The valleys are mostly covered by fruit trees of anthropogenic origin.

The collection site is a well-lit, windswept, open area with gently sloped terrain with grass and rocks. The stream is mostly surrounded by fruit trees. *Quercus, Populus* and *Rosa* trees are dominant in the vicinity, with *Salix* and *Carpinus* occasionally present. The climate is characterized by cold, snowy winters and hot, dry summers. Mean annual temperature is16. 6°C. The mean annual rainfall is about 380.6 mm, with a precipitation range of 206–633 mm. Mean annual humidity is 62% (Akman 1999).

Vouchers are stored in the herbarium of the Biology Department, Kazım Karabekir Education Faculty, Ataturk University, Erzurum, Turkey (ATAKKEF).

Species

Diplotomma hedinii (H. Magn.) P. Clerc & Cl. Roux, (as "*hedinianum*") Cryptogamica Helvetica 19: 292. 2004.

Specimen examined: **TURKEY. Erzincan:** Kemaliye, Avaz place, Avukatın çeşmesi (Lawyer's spring), 38°28'28"N, 39°13'02"E, on calcareous rock, 1375 m, 15.06.2009, ATAKKEF 450.

Thallus white or pale to dark grey, thin or thick, often wide-spreading, smooth or rimose-cracked or sometimes granular, ± determinate; prothallus absent. Apothecia 0.3–0.8(–1.5) mm diam.; disc flat at first, soon ± convex, often pruinose; thalline exciple sometimes present, white, sometimes ± crenulate; true exciple inconspicuous; epithecium brown; hymenium 45–75 µm tall, colourless. Asci 55(–65) × (10–)17(–24) µm. Ascospores 15–20 × 7–11µm, (1–)3-septate, not longitudinal septa, frequently curved and mostly submuriform at maturity. Thallus C–, K–, KC–, Pd–, UV–.

Diplotomma hedinii is mainly a temperate species of exposed calcareous rocks and sandstone outcrops in more or less sunny conditions. Previously known from Africa, Asia, Europe, and Central and North America; new to Turkey and the Middle East.

Remarks—*Diplotomma hedinii* is similar to *D. alboatrum,* but *D. alboatrum* has 15–24 × 8–10 µm ascospores with longitudinal septa and which are less frequently curved.

Lecanora valesiaca (Müll. Arg.) Stizenb., Ber. Thätigk. St. Gallischen Naturwiss. Ges. 81: 342.1882

Specimens examined: **TURKEY. Erzincan:** Kemaliye, Mezbanbaşı place, 6.5 km SW of Sırakonak, 38°25'54"N, 39°11'15"E, on siliceous rock, 1851 m, 10.06.2009,

ATAKKEF 453; 900 m SW of Kekikpınarı, 38°27'30"N, 39°08'37"E, on siliceous rock, 1275 m, 10.06.2009, ATAKKEF 456; Geşo Boğazı place, 1700 m NE of Yeşilyamaç, 38°34'00"N, 39°16'15"E, on siliceous rock, 1682 m, 11.06.2009, ATAKKEF 455; Avaz place, Avukatın Çeşmesi (Lawyer's Spring), 38°28'28"N, 39°13'02"E, on siliceous rock, 1375 m, 15.06.2009, ATAKKEF 452; vicinity of Çitköy, 38°36'22"N, 39°06'45"E, on siliceous rock, 1030 m, 12.07.2009, ATAKKEF 454.

Thallus up to 3.0 cm diameter, ± white, rosettes, pruinose, flat, frequently slightly undulate margins, yellow-greenish under the pruina, centrally areolate; thallus lobes rounded, older lobes not hollow; marginal lobes, 0.5–1.1 mm wide, (0.9–)1.0–2.0(–2.1) mm long, flattened, bifurcate and verrucose; upper surface, pruinose., Apothecia sessile to adnate, in the centre of the thallus 0.3–1.0(–1.3) mm diam., discs, yellow-brown light brown, pruinose, convex at maturity; margins thick, entire, covering the discs at first, thin, curved at maturity; epithecium brown; hypothecium pale yellow; paraphyses mainly cylindrical, unbranched, apices swollen; asci cylindrical-clavate, 8-spored, (25–)32–40(–42) × (11–)12–14(–15) µm; ascospores oval-ellipsoid, (8–)9–12 × 4–6(–7) µm. Thallus and medulla K–, C–, KC+ yellow, PD-.

Lecanora valesiaca grows on sloping faces of lime-poor, hard, base-rich rocks containing some calcium in warm-dry situations, and above all on south-facing exposed sites in the interior alpine dry valleys. Previously known from Asia, Europe, and North America; new to Turkey and the Middle East.

Remarks—*Lecanora valesiaca* is very similar to *L. freyi,* but *L. valesiaca* has thin lobes, a thicker margin, and verrucose lobe ends while *L. freyi* has thick lobes, a thin margin, and smooth lobe ends. Although both species possess similarly sized apothecia and ascospores, apothecia with incurved margins and yellow brown discs will separate *L. valesiaca* from *L. freyi*, characterized by apothecia with entire margins and flat, blackish green discs. The incurved apothecial margins, verrucose lobe ends, and pruinose thallus also distinguish *L. valesiaca* from *Protoparmeliopsis muralis* var. *muralis.*

Xanthoria weberi (S.Y. Kondr. & Kärnefelt) Aptroot, Herzogia 22: 151 (2009)

Specimen examined: **TURKEY. Erzincan:** Kemaliye, 1200 m, SE of Dutluca, 38°37'25"N, 39°07'42"E, on calcareous rock, 1165 m, ATAKKEF 451.

Thallus foliose, up to 4(–5) mm wide, forming small thalli, adnate to loosely adnate. Lobes horizontal to somewhat ascending, dorsiventral, flat, 0.3–0.5 mm wide, lobe tips often incurved, outermost parts of mature lobes ± truncate. Upper surface yellow to orange, smooth. Lower surface white. Cortex layers paraplechtenchymatous. Photobiont trebouxioid, green, unicellular. Medulla reticulate, of short cells. Rhizines mostly present (abundantly in a few specimens seen), white, yellow when exposed, attached or free. Soralia in lobe tips, often forming small bird nest soralia, soredia yellow. Apothecia rare, 0.7–2.0 mm wide. Ascospores 12.5–14 × 7–7.5 µm, septum 2.8–4.5 µm thick. Pycnidia

laminal, common, orange. Conidia bacilliform, 3.3–3.5 µm long. Cortex and apothecia K+ purple, C–, PD–, I–; medulla K–, C–, PD–, I–.

X. weberi grows in dry habitats on a variety of substrates, both bark and rock as well as on man-made substrates such as concrete and tombstone. Previously known from Europe and North America; new to Turkey and Asia.

Remarks—*Xanthoria weberi* resembles *X. gallowayi, X. oregana,* and *X. fulva. Xanthoria weberi* has shorter and slightly wider lobes with more incurved lobe tips than *X. gallowayi*, and where *X. weberi* sometimes has apothecia and frequently conspicuous pycnidia, no apothecia and pycnidia have been noted for *X. gallowayi* (Kondratyuk & Kärnefelt 1997). *Xanthoria weberi* has longer lobes and a brighter yellow pigmented upper surface than *X. fulva,* from which it is also distinguished by its dichotomously branched lobes, with more distinctly truncate lobe tips and relatively long internodes. The small bird nest-like soralia that occur mostly in the lobe tips and narrower, more regular dichotomously branched lobes in *X. weberi* help to differentiate it from *X. oregana.*

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First report of *Conidiobolus coronatus* in Turkey

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Abstract *Conidiobolus coronatus* (*Entomophthorales, Zygomycota*) was isolated from infected specimens representing an *Issus* sp. (*Issidae, Hemiptera*) collected from the Trabzon province of Turkey. The species, which represents a new record for the Turkish mycoflora, is described briefly and illustrated.

Key words insect, entomopathogenic fungi, hazelnut

Introduction

Members of the widespread order *Entomophthorales* (*Zygomycota*) are predominantly pathogens of insects and mites (Pell et al. 2001). Species of *Conidiobolus*, most notably the ubiquitous *C. coronatus*, are recorded as widespread soil saprophytes utilising a variety of substrates, including plant detritus, living plants, different dead arthropods and the fruiting bodies of other fungi in various regions of the world (MacLeod & Müller-Kögler 1973, Keller 1987, Sajap et al. 1997, Dromph et al. 2001, Laxman et al. 2005, Manning et al. 2007, Comerio et al. 2008). *Conidiobolus coronatus*, mainly tropical strains, is known to cause disease in both insects and humans (Ribes et al. 2000, Prabhu & Patel 2004); the disease has been named rhinophycomycosis, rhinophycomycosis entomophthorae, rhinoentomophthoromycosis, and conidiobolomycosis (King 1979, Ochoa et al. 1996, Yang et al. 2010).

Material & methods

Dead insects collected in 2008 from a hazelnut orchard in in the Black Sea region of Turkey were cultured for entomopathogenic fungi. After the cadavers were washed in a solution of 2% sodium hypochlorite for 1 min, they were dried on filter paper. After transfer to Petri dishes containing 20 ml of PDA, the cadavers were incubated at 25°C for 1 week with high humidity (80 + 10% rh). Colonies of filamentous fungi

emerging from each cadaver and identifiable as the genus *Conidiobolus* were transferred to PDA and identified to species using the relevant literature (Emmons & Bridges 1961, Prasertphon 1963, MacLeod & Müller-Kögler 1973, King 1979, Keller 1987, Humber 1997, Hatting et al. 1999, Toledo et al. 2007, Comerio et al. 2008). After identification, all isolates were deposited in the fungal collection of Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum-Turkey.

Results

The description and illustration of *Conidiobolus coronatus* given below are based on the Turkish collections of the material. This is the first report of *C. coronatus* from Turkey.

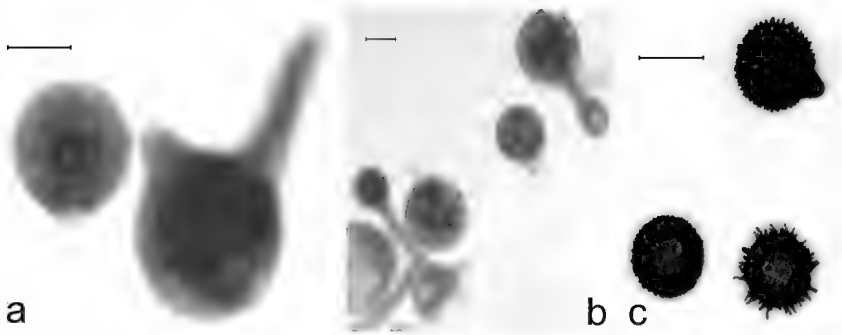

FIGURE 1. *Conidiobolus coronatus:* A primary conidium and germinating primary conidium; B primary conidia and secondary conidia produced on germ tube arising from primary conidia; C villose conidia may be the equivalent of resting spores in this species. Bar 20 µm.

Conidiobolus coronatus (Costantin) A. Batko, *Entomophaga,* Mem. Hors. Ser. 2: 129. 1964. FIG. 1

COLONIES (PDA) expanding, hyaline, soon with irregular radial blooms; at 25°C reaching 85 mm diam. after 2 days; PRIMARY CONIDIA (FIG. 1A) globose, with more prominent basal papilla tapering toward obtuse apex; variable in size, (23.2–)32.1(–46.8) × (18.1–)24.6(–35.1) µm; basal papilla prominent with pointed apex, forcibly discharged; SECONDARY CONIDIA (FIG. 1B) forming singly and forcibly discharged or (more commonly) producing many forcibly discharged secondary microconidia on short germ tubes arising from primary conidia; CONIDIOPHORES simple, unbranched; VILLOSE CONIDIA (resting spores; FIG. 1C) resembling primary conidia but covered with villose appendages (unique to this species), (19.9–)25.8(–34.8) µm diam.

SPECIMEN EXAMINED: On cadavers of *Issus* sp. (*Issidae, Hemiptera*). **TURKEY: TRABZON PROVINCE, Of district**, *Corylus avellana* L. (*Corylaceae*) orchards, 40°51'30"N, 40°16'00"E, alt. 160 m, VI-VIII 2008, coll. K AK (KA 123).

Discussion

Conidiobolus coronatus, originally isolated from a culture of *Agaricus campestris* L. (possibly derived from a dead insect hidden between the lamellae), was described in 1897 as *Boudierella coronata* Costantin (MacLeod & Müller-Kögler 1973). Since then it has been isolated from numerous and diverse sources (MacLeod & Müller-Kögler 1973, King 1979, Keller 1987, Sajap et al. 1997, Dromph et al. 2001, Laxman et al. 2005, Manning et al. 2007, Comerio et al. 2008). Utilization of the species as a biological control is limited by its potential to cause human disease (King 1979, Ochoa et al. 1996, Ribes et al. 2000, Prabhu & Patel 2004).

Diameters of both the primary and villose conidia measured in this study fell within the ranges observed by Emmons & Bridges (1961; primary conidia: 36–44 µm), Prasertphon (1963; primary: 25–61 µm; villose: 8–42 µm), Keller (1987; primary: 37–74 µm; villose: 16–42 µm), Hatting et al. (1999; primary: 41.5–66 × 30–48 µm), Toledo et al. (2007; primary: 17.6–39.5 × 24.7–29.6 µm; villose: 19.8–24.7 µm), and Comerio et al. (2008; primary: 30–38 µm).

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Carbonea, Gregorella, Porpidia, Protomicarea, Rinodina, Solenopsora, and *Thelenella* lichen species new to Turkey

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ABSTRACT Seven species of lichenized fungi (*Carbonea vorticosa, Gregorella humida, Porpidia tuberculosa, Protomicarea limosa, Rinodina turfacea, Solenopsora holophaea,* and *Thelenella modesta*) are new to Turkey. *Gregorella humida* is also new to Asia. For each a short description is presented.

KEY WORDS biota, Giresun, Ordu

Introduction

Since John (2004) listed 361 papers referring to lichens from Turkey, many additional studies have been carried out on Turkish lichen biota (e.g., Aptroot & Yazıcı 2009, Aslan et al. 2005, Candan & Özdemir Türk 2008, Çobanoglu et al. 2008, Halıcı & Aksoy 2006, Kınalıoglu 2010, Öztürk & Güvenç 2010, Oran & Öztürk 2010, Yazıcı & Aptroot 2008). However, there are still a high number of records for lichen species yet to be added. This contribution reports further first records of species new to Turkey.

Materials & methods

The collections were identified with various lichen guides (Brodo et al. 2001, Purvis et al. 1992, Smith et al. 2009, Wasser & Nevo 2005, Wirth 1995). They are deposited in the herbarium of the Faculty of Science and Arts, Giresun University, Giresun, Turkey. The collector and collection number are given in parentheses after the locality details.

Species recorded

Carbonea vorticosa (Flörke) Hertel

A detailed description is provided by Chambers et al. (2009).

SPECIMEN EXAMINED: **TURKEY. ORDU**: Kumru, Sarıçiçek high plateau, 40°45'28"N, 37°12'51"E, 1521 m, 16 Jul. 2005, on siliceous rock, (herb. Kınalıoğlu 1802).

Thallus brownish, subsquamulose to eroded-dispersed. Apothecia 0.2–0.7 mm diam., scattered; disc black, concave, rarely plane. Epihymenium dark blue. Ascospores 7.5–12 × 3.7–5 µm, colourless, simple. Thallus C–, K–, KC–, PD+ orange.

Carbonea vorticosa is often overlooked. In other parts of its range, it grows on siliceous rock, boulders, and walls in upland to high montane habitats. In Turkey, it was collected only from siliceous rock in a sun exposed area.

Known from Europe, Asia, Australasia, Antarctica, North America and South America (Andreev 2003). New to Turkey.

Gregorella humida (Kullh.) Lumbsch

Detailed descriptions are provided by Lumbsch et al. (2005) and Woods (2009).

Specimen examined: **TURKEY. Ordu**: Ünye, the top of Ünye castle, 40°05'46"N, 37°14'22"E, 245 m, 07 Aug 2004, on siliceous rock, (herb. Kınalıoğlu 1800).

Thallus bluish grey, consisting of roundish goniocysts. Photobiont *Nostoc*. Apothecia 0.2–0.7 mm diam., numerous, roundish; disc convex, colourless when young, red-brown when mature, true exciple yellowish. Hymenium I–. Ascospores colourless, 12.5–20 × 6.5–10 µm. Thallus C–, K–, KC–, PD–.

Gregoriella humida is probably much overlooked. In other parts of its range, it is a pioneer lichen, growing on well drained acidic to somewhat basic, ± clayey soil exposed on steep banks such as in disused railway cuttings, on tipped material on roadsides and on the ground in old lead mines. In Turkey, it was collected only from siliceous rock.

Known from Europe (Cezanne et al. 2003). New to Asia.

Porpidia tuberculosa (Sm.) Hertel & Knoph

A detailed description is provided by Fryday et al. (2009).

Specimen examined: **TURKEY. Giresun**: Keşap, Değirmenağzı village, sea shore, 40°58'20"N, 38° 37'23"E, 0 m, 11 Apr. 2010, on siliceous rock, (herb. Kınalıoğlu 1809).

Thallus white to pale blue-grey, sometimes rusty red in parts; prothallus dark brown-black; soralia 0.1–0.8 mm diam., round to irregular; soredia pale grey or blue-grey. Apothecia not observed. Medulla and soralia C–, K–, KC–, I+violet, PD–.

In other parts of its range, *P. tuberculosa* is a common species found on siliceous rocks, walls, pebbles, and rarely on worked timber. In Turkey, it was collected only from siliceous rock at the sea shore.

Known from North America, South America, Asia, Europe, Macaronesia (Fryday et al. 2009). New to Turkey.

Protomicarea limosa (Ach.) Hafellner

A detailed description is provided by Coppins (2009).

Specimen examined: **TURKEY. Giresun**: Dereli, E of Kuzu gölü high plateau, 40°34'42"N, 38°29'21"E, 2200 m, 09 Aug. 2008, on dead grass, (herb. Kınalıoğlu 1803).

Thallus greyish. Apothecia 0.25–0.9 mm diam., black. Epihymenium and hymenium greenish, K–; hymenial gel I+red; hypothecium K+orange. Ascospores colourless, narrowly ellipsoid, 10–15 × 3.5–5 µm. Thallus C–, K–, KC–, PD+ orange-red.

This rare species is found on acid soil, over mosses or humus mostly above 900 m. In Turkey, *P. limosa* was collected only from dead grass in sun exposed areas at high elevation.

Known from Europe and North America (Smith et al. 2009). New to Asia.

Rinodina turfacea (Wahlenb.) Körb.

Detailed descriptions are provided by Brodo et al. (2001) and Thomson (1997).

Specimen examined: **TURKEY. Giresun:** Dereli, E of Kuzu gölü high plateau, 40°34'42"N, 38°29'21"E, 2200 m, 09 Aug. 2008, on moss, (herb. Kınalıoğlu 1808).

Thallus thick, pale brownish or grey. Apothecia abundant, 0.5–1.8 mm diam.; disc dark brown to black; concolourous with thalline exciple. Ascospores thick-walled, brown, *Physcia*-type, 22.5–35 × 10–15 µm. Thallus C–, K–, KC–, PD–.

Rinodina turfacea is found on soil, moss, decaying vegetation, peat and wood in arctic or alpine sites. In Turkey, it was collected only from moss in sun exposed areas at high elevation.

This species is known from Europe, Asia, North America, and South America (Brodo et al. 2001, Kotlov 2008, Redchenko et al. 2010). New to Turkey.

Solenopsora holophaea (Mont.) Samp.

A detailed description is provided by Gilbert at al. (2009).

Specimens examined: **TURKEY. Ordu:** Fatsa, Yalıköy, sea shore, 0 m, 13 Jul. 2005, on siliceous rock, (herb. Kınalıoğlu 1798). **Giresun:** Dereli, E of Kuzu gölü high plateau, 40°34'42"N, 38°29'21"E, 2200 m, 09 Aug. 2008, on soil, (herb. Kınalıoğlu 1799).

Thallus squamulose to 3 mm wide, upper surface red-brown when dry and olive green when wet. Apothecia 0.5–1.2 mm diam., numerous, disc dark reddish brown to black. Ascospores 2-celled, colourless, 12.5–17 × 4 µm in size. Thallus C–, K–, KC–, PD–.

In other parts of its range, this species grows on soil and soft rocks chiefly in slightly sheltered clefts on vertical cliffs or old walls at the coast. In Turkey, it was collected from siliceous rock at the sea shore and from soil in sun-exposed areas at high elevation.

Solenopsora holophaea is known from Africa, Asia, Europe, Macaronesia, North America, and South America (Güvenç & Öztürk 1999, Smith et al. 2009). New to Turkey.

Thelenella modesta (Nyl.) Nyl.

Detailed descriptions are provided by Smith et al. (2009) and Wasser & Nevo (2005).

Specimen examined: **TURKEY. Ordu:** Kumru, Ericek high plateau, 40°53'35"N, 37°13'48"E, 1120 m, 16 June 2005, on *Juglans regia* L. (*Juglandaceae*), (herb. Kınalıoğlu 1813).

Thallus pale yellowish-grey. Perithecia 0.3–0.8 mm diam., rounded, sparse. Ascospores muriform, colourless, 25–42 × 10–17 µm. Thallus C–, K–, KC–, PD–.

Elsewhere in its range growing on ± rough bark of wayside or parkland *Fraxinus,* twigs of *Crataegus*, and smooth bark of *Quercus agrifolia* and *Juglans californica*. In Turkey, we recorded it from the trunk of *Juglans regia.*

Thelenella modesta is known from Europe, North America, Asia, Africa, and Australia (Smith et al. 2009, Wasser & Nevo 2005). New to Turkey.

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Type studies of *Russula* species described by W.A. Murrill, 1. *R. roseiisabellina, R. sericella,* and *R. obscuriformis*

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Abstract The study of the North American russulas remains severely handicapped by the lack of sufficient microscopic detail on approximately one third of the described taxa. In this contribution, microscopic features are examined and illustrated in detail for three of Murrill's type specimens. The authors reinterpret these data in combination with the macroscopic features supplied in the original diagnoses and propose a more appropriate infrageneric placement of the taxa: (i) *R. roseiisabellina* is not close to *R. sericeonitens* (subgenus *Russula*) as suggested in Murrill's protologue, nor is it a synonym of *R. rimosa* (subgenus *Heterophyllidia*) as suggested by Hesler it is a typical member of the *R. foetens* group (subgenus *Ingratula*); (ii) *R. sericella* is absolutely unrelated to *R. obscuriformis* as suggested in Murrill's protologue and probably fits within subgenus *Russula* or *Tenellula*; and (iii) *R. obscuriformis* is similar to the recently described *R. texensis* in *Russula* sect. *Xerampelinae*, but new collections are needed to verify whether it possesses the typical field characters of this group.

Key words Florida, micromorphology, *Russulaceae*, taxonomy

Introduction

William Murrill (1869–1957) was without any doubt one of the most colorful and influential personalities among 20th century mycologists in America (Weber 1961). His prolific description of new taxa has given him a very controversial reputation, also in the case of *Russula* Pers., and Murrill surpasses by far his fellow mycologists with a total of 111 newly described *Russula* species, most collected near Gainesville, Florida (Buyck 2007).

As a professional mycologist, Murrill started his career at the New York Botanical Garden, where he was probably influenced and stimulated towards description of new taxa by the nomenclatural views and ambitions of the newly imposed "American Code for nomenclature" at the New York Botanical

Garden (Weber 1961). Murrill usually published his new russulas without any critical revision or discussion of related or similar taxa and without placing or grouping them into a subgeneric classification scheme. His descriptions were very short and mainly oriented towards field characters at a time when the study of the microscopic features of European *Russula* (Maire 1910, Melzer 1934) had already reached the degree of precision that is still in use today. Judging from published synonymies (Bills 1984, Bills & Miller 1984) and our own experience with his collections, it is therefore not surprising that Murrill had probably difficulties in recognizing his own taxa (see commentary under *R. roseiisabellina* below for example).

Hesler (1960, 1961) and Singer (1947, 1958) were the only mycologists who ever tried to add something more substantial to Murrill's original diagnoses. Hesler published very short notes on microscopic features for many of Murrill's types, but even the kind of precision Hesler added for spores and elements of the pileipellis is still largely insufficient to allow for good interpretations and comparisons with modern descriptions of *Russula*. More recently, a few other mycologists (e.g., Bills 1984, Bills & Miller 1984, Kibby & Fatto 1990) have studied at least some of Murrill's collections but mostly without publishing the features they examined on Murrill's types. In a few instances, these re-examinations eventually resulted in proposed synonymies or different interpretations for some of Murrill's taxa.

With taxonomic expertise on *Russula* having literally vanished in North America over the past decennia (Buyck 2007) and in the absence of precise data on Murrill's types, the study of the North American russulas remains severely handicapped by the lack of sufficient microscopic detail on approximately one third of the described russulas on the continent.

This paper is the second of a series of detailed and illustrated microscopic revisions of Murrill's taxa in an effort to promote and stimulate rapid progress in the study of American *Russula* (see Adamčík & Buyck 2010). We also try to place the examined species in the latest available (mainly European) classifications of the genus. And even if such a placement remains very tentative in the absence of more precise data on fresh material, our observations suggest in most cases very different affinities for most of the examined species.

One character shared by nearly all taxa in this contribution concerns the color change on bruising or on drying: nearly all examined taxa supposedly exhibit a distinct browning of at least part of the fruit bodies: *R. roseiisabellina* (Murrill 1943) has lamellae "becoming brownish where bruised", *R. sericella* (Murrill 1945) was described with a stipe "becoming pale-brownish when handled". Finally, we also include here *R. obscuriformis* (Murrill 1945), which was considered to be a synonym of *R. sericella* by Singer (Singer in sched.).

Materials & methods

Micromorphological characters were observed in Olympus CX-41 and Nikon Eclipse E400 microscopes using an oil-immersion lens at a magnification of 1000×. All drawings of microscopical structures – except of spores – were made with 'camera lucida' using a Nikon Y-IDT drawing attachment at a projection scale of 2400×. Contents of hymenial cystidia and pileocystidia in the illustrations are indicated schematically, except for a single element where contents are indicated as observed in Congo red preparations from dried material. Spores on the lamellae were observed in Melzer's reagent. All other microscopic observations were made in ammoniacal Congo red, after a short aqueous KOH pre-treatment to improve tissue dissociation through gelatinous matrix dissolution. All tissues were also examined for the presence of ortho- or metachromatic contents or incrustations in cresyl blue as explained in Buyck (1989).

Spores were scanned with an Olympus Artcam camera and measured using Quick Micro Photo (version 2.1) software. Enlarged scanned pictures of spores were used for measuring with an accuracy of 0.1 µm and for drawing. Q gives length/width ratio of the spores. Measurements exclude ornamentation. Statistics for measurements of microscopical characters are given as mean value (boldface) plus/minus standard deviation and are based on 30 measurements. Values in parentheses give measured minimum or maximum values. An estimate for spore ornamentation density is given following Adamčík & Marhold (2000).

Names for infrageneric taxa follow the classification system proposed by Romagnesi (1985, 1987).

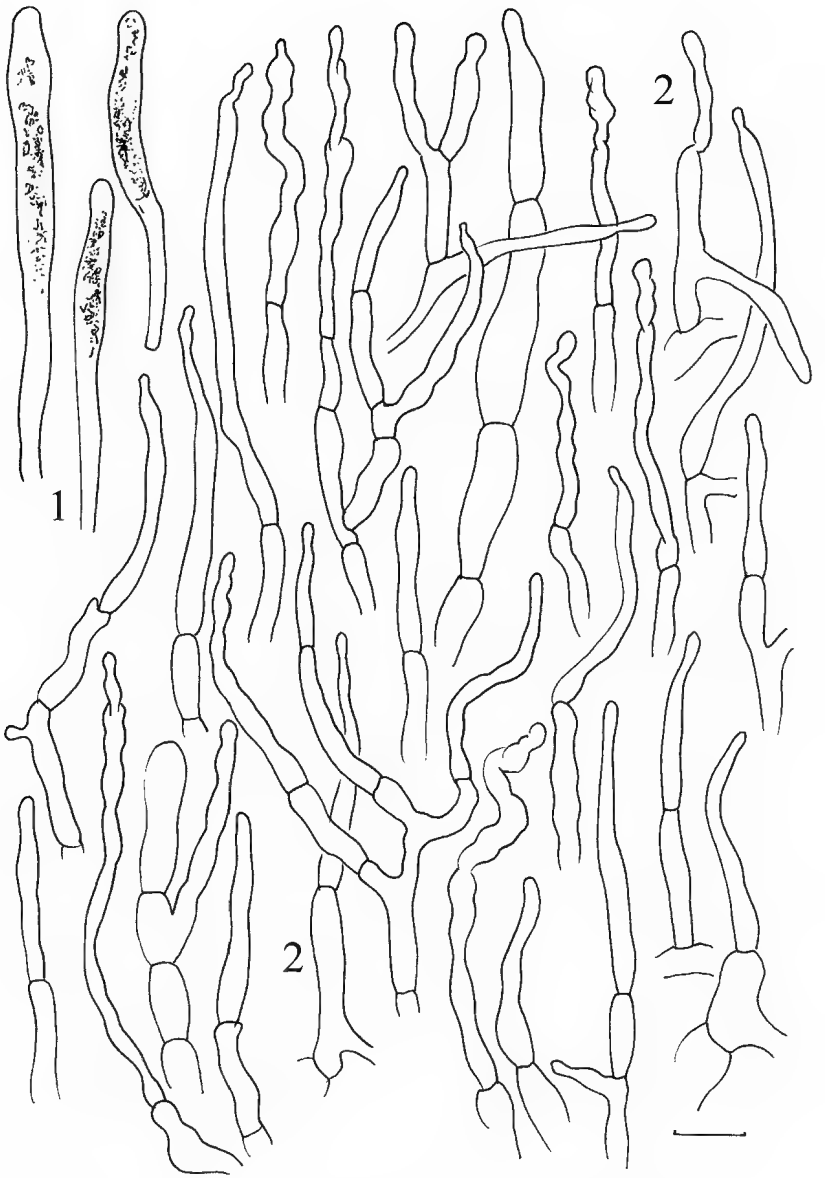
Taxonomy

Russula obscuriformis Murrill, Lloydia 7(4): 312. 1945. FIGS 1–6

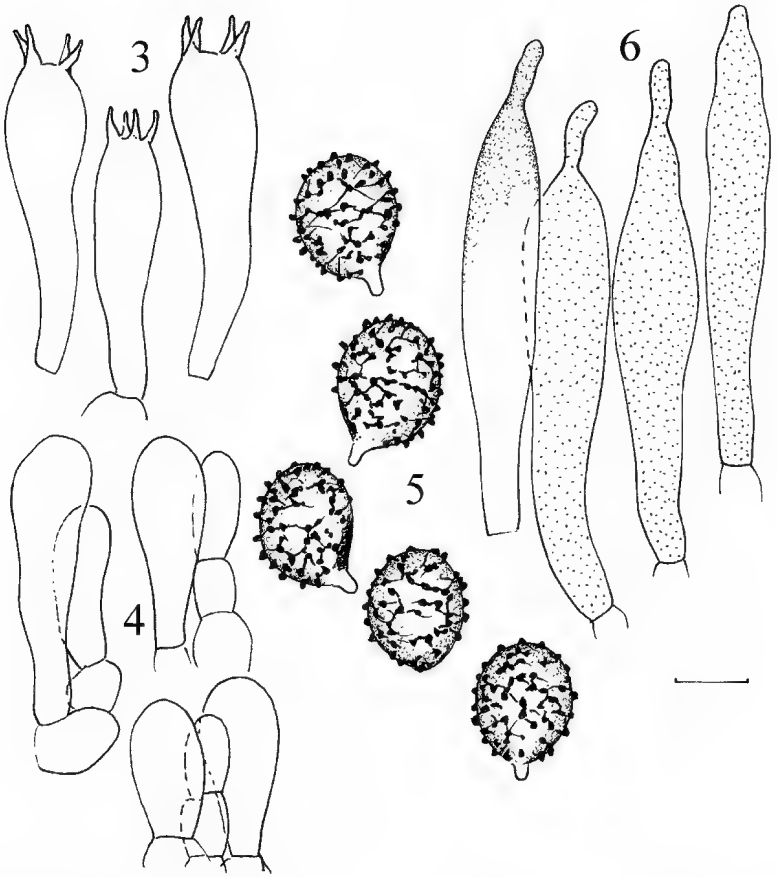
ORIGINAL DESCRIPTION AND TYPIFICATION — Pileus convex to slightly depressed, solitary, 8 cm broad; surface dry, smooth, at length glabrous, dark-roseous with bay center, darker on drying, margin even or slightly striate, entire, peeling readily; context thin, white, discolored on drying, odorless, mild; lamellae adnate, mostly equal, few forked, broad, medium distant, entire, white; spores subglobose to broadly ellipsoid, finely tuberculate, stramineous in mass, about 8 µm long; cystidia none; stipe equal, smooth, glabrous, white, 5 × 2 cm.

Type collected by W. A. Murrill under an oak at LaCrosse, Alachua Co., Fla., July 13, 1938 (F 15536). Suggesting *R. obscura* Romell but differing in color and spore color. The gills in dried specimens are distinctly gray. [Holotype at FLAS sub nr. F15536].

MICROSCOPIC FEATURES — SPORES broadly ellipsoid, (7.3–)7.7–**8.1**–8.4(–8.7) × (6.2–)6.4–**6.7**–7(–7.4) µm, Q = (1.11–)1.15–**1.2**–1.25(–1.27); ornamentation rather dense, subreticulate to reticulate, composed of conical to hemispherical, often large, strongly amyloid warts, ca (3–)5–7(–8) warts in a 3 µm diam. circle, measuring 0.5–0.7 µm high, interconnected by fine line connections (2–6 in a circle) or fused in ridges (0–4 fusions in the circle); suprahilar plage amyloid. BASIDIA (32–)37.5–**40.5**–43.5(–45) × (10–)11–**12**–13(–13.5) µm, 4-spored, clavate. SUBHYMENIUM pseudoparenchymatic. LAMELLAR TRAMA mainly composed of large spherocytes. HYMENIAL CYSTIDIA widely dispersed, less



FIGS. 1 2. *Russula obscuriformis* (holotype). 1. Pileocystidia, with contents indicated in one element as seen in Congo red. 2. Hyphal terminations near cap margin. Scale bar = 10 µm.



FIGS. 3–6. *Russula obscuriformis* (holotype). 3. Basidia. 4. Basidiola. 5. Spores in Melzer's reagent. 6. Hymenial cystidia with contents indicated in one element as seen in Congo red.
Scale bar = 5 µm for spores, 10 µm for the other elements.

than 500/mm² and very difficult to observe except near gill edge, measuring ca. 56–80 × 9–14 µm on sides, clavate to fusiform-pedicellate, mucronate-appendiculate, thin-walled, with few and ill-defined, SV-negative contents that are not strongly refringent in KOH. MARGINAL CELLS not differentiated. PILEIPELLIS orthochromatic in cresyl blue, without incrustations, not sharply delimited from the underlying spherocytes of the context, vaguely divided in a rather poorly gelatinized subpellis and a more dense suprapellis of intricate to ascendant hyphae, with poorly differentiated pileocystidia. Hyphal endings thin-walled and easily collapsing, near margin with terminal cells measuring (19–)24.5–**34.2**–44(–55) × (2.5–)3–**3.2**–4(–4.5) µm, slender, attenuated or

cylindrical, with apex often more or less abruptly, irregularly or repeatedly constricted and only 1.5–2.5 µm diam., occasionally clavate; subapical cells shorter and often somewhat larger in diam., often branched. Pileocystidia scarce and indistinct, single-celled, arising from the subpellis, cylindrical to subclavate, ca. 4–5.5(7.5) µm wide, with poorly differentiated, granular contents that do not react to sulphovanillin. CLAMP CONNECTIONS absent in all parts.

COMMENTARY — *R. obscuriformis* differs from all other species discussed in this paper by the spores that are "stramineous in mass" – an observation that confirms at least that Murrill did distinguish between pale and darker spore prints in *Russula*. It is therefore difficult to understand why Singer synonymizes this species with *R. sericella* (having a white spore print), which has indeed a completely different pileipellis under the microscope.

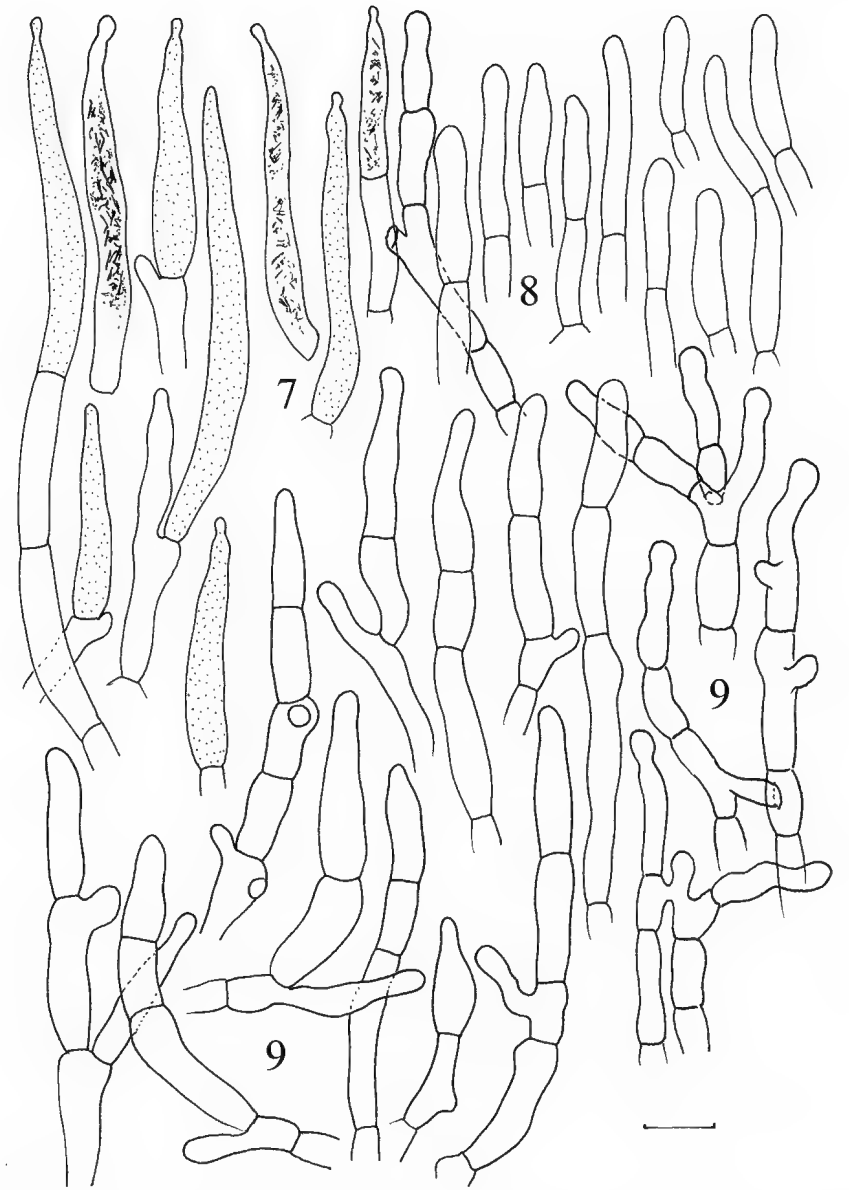
The discoloring context should, in our opinion, be interpreted as 'graying' context because of the comparison to "*R. obscura* Romell" (= *R. vinosa* Lindblad) and the mention of the distinctly grayish lamellae on drying. This graying context, together with the odorless flesh and the very prominent reticulation of the spores, is a combination of characters that does not suggest a place in *Russula* sect. *Xerampelinae* (Singer) Jul. Schäff. However, the recently described *R. texensis* (Buyck et al. 2008) is a fishy *Russula* with grayish-brownish discoloring context and a weak fishy smell. It has an overall similar color and also similar features of spores and pileipellis. The two taxa differ nevertheless by the much more pronounced reticulation of the spores in *R. obscuriformis* and the very scarce, smaller and hardly prominent pileocystidia. The spore print color of *R. texensis* was noted as 'pale' on the gills (a sufficient spore print was not obtained). A dark cream to pale ochre spore print may therefore still be possible and could perhaps match the 'stramineous' color noted by Murrill for *R. obscuriformis*. It can therefore not be excluded for the moment that Murrill's species is a good member of sect. *Xerampelinae*.

The use of the synoptic key by Kibby & Fatto (1990) is quite frustrating in the case of *R. obscuriformis* since the interpretation of many features (in particular cap color, spore print color, peeling and bruising), does not allow for unambiguous coding and therefore does not lead to any reliable identification.

Russula roseiisabellina Murrill, Lloydia 6(3): 216. 1943. FIGS 7–13

ORIGINAL DESCRIPTION AND TYPIFICATION — Pileus convex to depressed, 4.5–6 cm broad; surface glabrous, slightly viscid, the cuticle not readily separable, rosy-isabelline, margin entire, somewhat striate; context rather thick, white, unchanging, sweet, odorless; lamellae adnate, narrow, close, entire, mostly equal, scarcely any forked, white to pallid, becoming brownish where bruised; spores white in mass, globose or subglobose, distinctly echinulate, 6–7 µm; stipe tapering downward, smooth, glabrous, white, unchanging on drying but brownish where bruised, 4–6 × 1–1.5 cm.

Type collected under an evergreen oak on the west shore of the Prairie, near Gainesville, Fla., June 19, 1938 (F 16365). Also collected under oaks at the Tung-oil

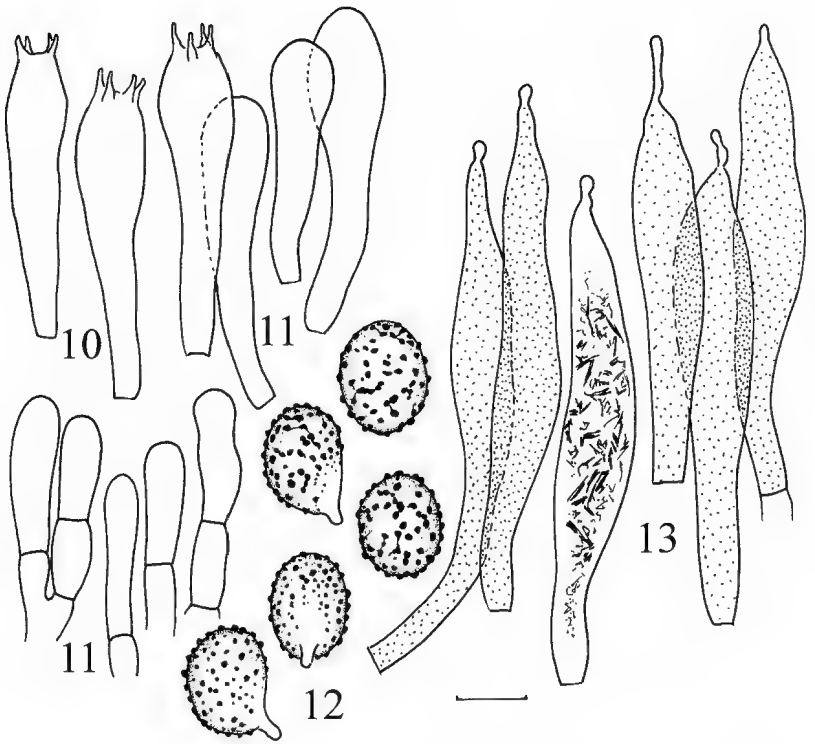


Figs. 7 9. *Russula roseiisabellina* (holotype). 7. Pileocystidia, with contents indicated in one element as seen in Congo red. 8. Hyphal terminations in cap center. 9. Hyphal terminations near cap margin. Scale bar 10 µm.

Mill, west of Gainesville, June 15, 1938, by Margaret Johnson (F 17209), and in the same locality, June 22, 1938, by West and Murrill (F 17210). This species seems to prefer soil with some lime in it. It is rare, and is found alone or, at most, two near together. [Holotype at FLAS sub nr. F16365].

MICROSCOPIC FEATURES — SPORES ellipsoid, (6.1–)6.7–**7.1**–7.6(–8) × (4.7–)4.8–**5.1**–5.4(–5.9) µm, Q = (1.18–)1.29–**1.39**–1.49(–1.54); ornamentation dens (with 7–10(–13) warts in a 3 µm circle), composed of low and obtuse, amyloid warts measuring 0.2–0.3 µm high, mostly isolated, with some very rare connectives or twinned warts (0–2 fusions in the circle) on some spores; suprahilar plage inamyloid. BASIDIA (35–)40–**42.6**–45(–46) × (9–)9.5–**10.3**–11(–11.5) µm, 4-spored, clavate. SUBHYMENIUM pseudoparenchymatic, small-celled. LAMELLAR TRAMA mainly composed of large spherocytes. HYMENIAL CYSTIDIA dispersed, often less than 600/mm² but more numerous on gill edge, measuring (35.5–)47.5–**56.4**–65.5(–70) × (7.5–)8.5–**10.2**–12(–14) µm, fusiform-pedicellate, mucronate-appendiculate, thin-walled, with SV-negative, crystalline-granular contents, that are strongly refractive in KOH. MARGINAL CELLS undifferentiated. PILEIPELLIS near the surface slightly metachromatic in cresyl blue, not sharply delimited from the underlying context, thin, vaguely divided in a poorly gelatinized subpellis of intermingled hyphae, and a suprapellis of more branched and intricate endings with many pileocystidia near the surface only. Hyphal endings thin-walled, often branched at the first or second subapical cell, overall slightly narrower and with shorter terminal cells in cap center compared to cap margin; terminal cells near margin measuring (12–)15.5–**21.6**–28(–42.5) × 4–**4.8**–5.5(–6) µm, subcylindrical or mostly somewhat narrowing upward, in cap center (12.5–)15.5–**19.8**–24(–29) × (3–)3.5–**3.8**–4(–5) µm, less narrowing but on the contrary more often slightly inflated at the tip and subcapitulate; subapical cells mostly equal in width, often branched. Pileocystidia present near surface only, numerous, unicelled, (23.5–)31–**42.1**–53(–68) × (3.5–)4.5–**5.2**–6(–6.5) µm with the largest one near the cap margin, narrow, fusiform to typically subulate with mucronate tips, thin-walled; contents granular-crystalline, often concentrated in upper half or middle portion of the cystidium, insensitive to sulfovanillin. CLAMP CONNECTIONS absent in all parts.

COMMENTARY — Because of the mention of the white spore print, the mild taste, and a weakly striate cap margin in the original description, this species keys out to *R.* subgen. *Heterophyllidia* Romagn. when using most keys for European russulas (Bon 1988, 2002a,b; Romagnesi 1985). Using the synoptic key of Kibby & Fatto, the easy and straightforward coding of features (FHKNPTV) leads directly to *R. decora* Shaffer. The latter species is a member of the *R. nigricans* group (*R.* subgen. *Compactae* (Fr.) Bon) and therefore absolutely not a close match for Murrill's type.



FIGS. 10–13. *Russula roseiisabellina* (holotype). 10. Basidia. 11. Basidiola. 12. Spores in Melzer's reagent. 13. Hymenial cystidia with contents indicated in one element as seen in Congo red.
Scale bar = 5 µm for spores, 10 µm for the other elements.

Our own observations on features of spores and particularly of pileipellis place *R. roseiisabellina* without any doubt in *R.* subgen. *Ingratula* Romagn. (perhaps close to *R. ventricosipes* Peck): the non-amyloid suprahilar spot, the slender, narrow, mucronate pileocystidia, the weak metachromatic reaction of the suprapellis elements in cresyl blue, and finally the spore ornamentation consisting of low, mostly isolated and obtuse warts are all well known features for other taxa in this group. The metachromatic reaction in cresyl blue and the type of spore ornamentation may also remind of *R.* subsect. *Cyanoxanthinae* Singer (*R.* subgen. *Heterophyllidia*) but the latter species group differs by a different and very characteristic type of pileocystidia and in having strongly incrusted, metachromatic hyphal ends, which are quite different from the ones observed here.

R. roseiisabellina therefore appears to be a good and perhaps rare species that does not correspond to any of the already described members of subgen. *Ingratula* in North America. Whether it is very characteristic in the field is unlikely: our examination of the second specimen identified and mentioned as such in the protologue by Murrill reveals a clearly different species of the *R. foetens*-group with very different spores.

Hesler (1960), who has published a "type-study" with some notes on the spores and pileipellis of *R. roseiisabellina,* describes a quite different type of pileipellis, reminiscent of *R. virescens* (Schaeff.) Fr., and concludes that Murrill's species is identical to *R. rimosa* Murrill, reducing the latter into synonymy. However, as the senior author has had access to Hesler's personal files and duplicate collections at TENN, it is evident that the disagreement between Hesler's and our own observations can be easily explained by the fact that Hesler never examined the type, but relied for his publication on a different, but misidentified specimen sent to him by Murrill.

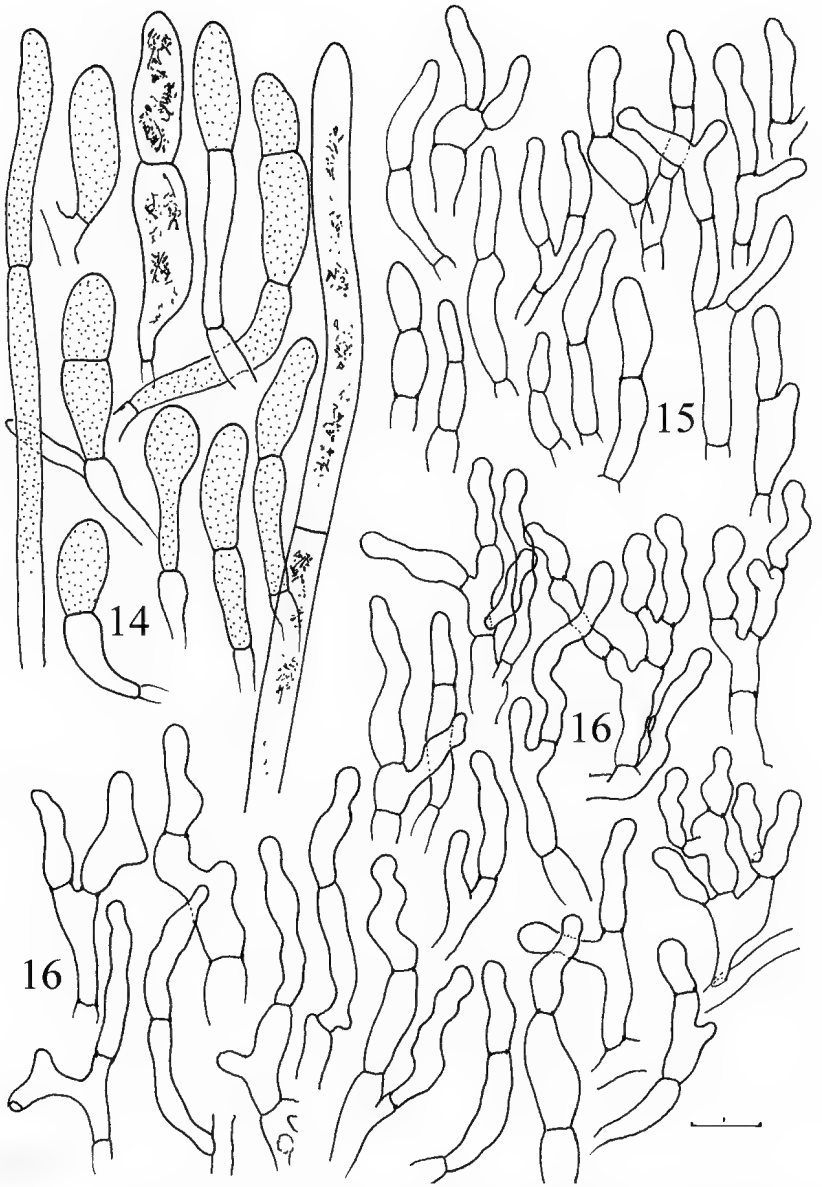
The essential features of *R. roseiisabellina* reside with the pinkish flush on the cap, the pale spore print (described as white, but given the imprecision at that period, a pale cream spore print can not be excluded), mild taste, browning context at least for the gills, and absence of a distinct smell. The importance of its association with *Quercus* on limy soil remains to be verified, but may help in separating it from small individuals of *R. ventricosipes,* a typical pine associate on sandy soil possessing equally a pinkish flush on the cap.

Russula sericella Murrill, Lloydia 7(4): 313. 1945. FIGS 14–21

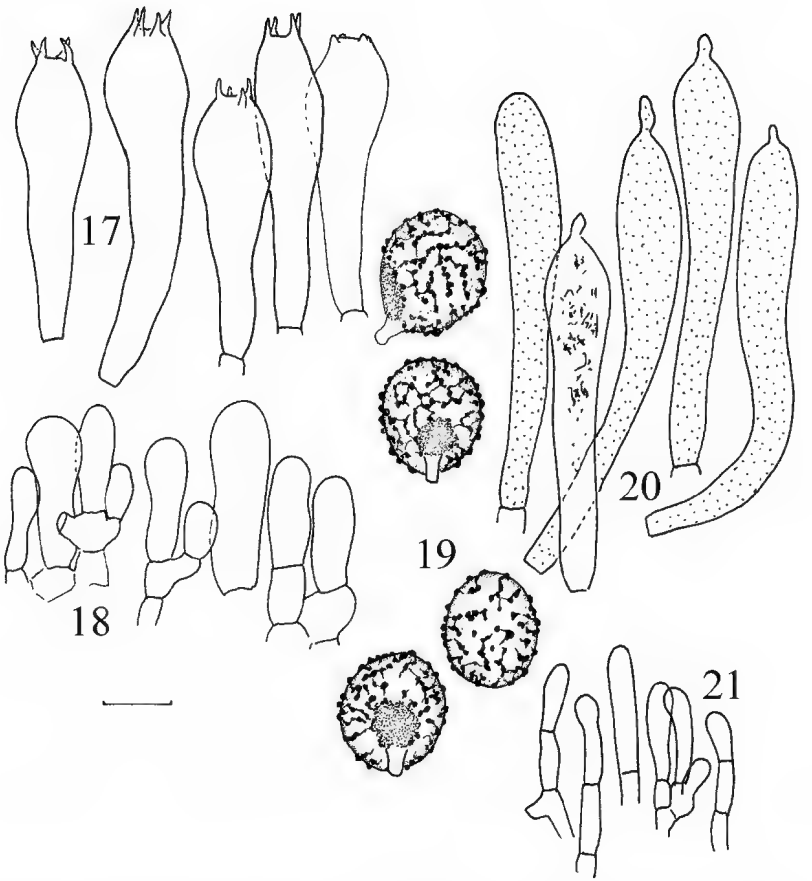
ORIGINAL DESCRIPTION AND TYPIFICATION — Pileus convex to depressed, 5–7 cm broad; surface dry, smooth, finely pruinose, vinosous on the disk, paler toward the margin, which is entire and very slightly striate at times; context white, unchanging, odorless, mild; lamellae adnate, many forked at the very base, few inserted, close, narrow, entire, white, gray when dried; spores globose or subglobose, plainly short-tuberculate, white in mass, 6–7 µm; cystidia none; stipe usually equal, stuffed, smooth, glabrous, white, becoming pale-brownish when handled, 5–7 × 1.5–2 cm.

Type collected by W. A. Murrill under a live-oak in Gainesville, Fla., June 1, 1938 (F 12075). Also collected several times under frondose trees in Alachua Co. and once in Clay Co., Fla. Closely related to Kauffman's *R. sericeonitens* but easily distinguished by the change in the color of the stipe. Spores tuberculate; not echinulate as in *R. xerampelina* Fr. [Holotype at FLAS sub nr. F12075].

MICROSCOPIC FEATURES — SPORES subglobose to broadly ellipsoid, (7.3–)7.5–**7.8**–8.1(–8.5) × (6.3–)6.5–**6.8**–7(–7.4) µm, Q = (1.07–)1.11–**1.16**–1.21(–1.35); with a dense subreticulate ornamentation (ca (7–)8–10(–11) elements in a 3 µm circle), composed of broadly conical, amyloid, warts measuring 0.4–0.6 µm high, with numerous line connections ((0–)2–5(–6) connections in a 3 µm circle) or locally fused in short or long ridges (0–4(–5) fusions in the circle); suprahilar plage amyloid. BASIDIA (37–)39.5–**42.2**–45(–48) ×



FIGS. 14 16. *Russula sericella* (holotype). 14. Pileocystidia, with contents indicated in one element as seen in Congo red. 15. Hyphal terminations in cap center. 16. Hyphal terminations near cap margin. Scale bar 10 μm.



Figs. 17–21. *Russula sericella* (holotype). 17. Basidia. 18. Basidiola. 19. Spores in Melzer's reagent. 20. Hymenial cystidia with contents indicated in one element as seen in Congo red. 21. Marginal cells of gill edge. Scale bar = 5 µm for spores, 10 µm for the other elements.

(10–)11–**11.9**–12.5(–13) µm, 4-spored, clavate. Subhymenium small-celled. Lamellar trama with many very large spherocytes. Hymenial cystidia on sides moderately numerous (1000–1500/mm²), hardly emergent, originating in the subhymenium, measuring (49–)52.5–**63.3**–74(–89) × (7–)8–**9.3**–10.5(–11) µm, mostly obtuse, occasionally mucronate or appendiculate (up to 4 µm long), thin-walled, with SV-negative contents. Marginal cells on edge of gills very small and slender, hardly differentiated, ca. 7–18 × 2.5–4 µm. Pileipellis orthochromatic in cresyl blue, without incrustations, not sharply delimited from the underlying large spherocytes of the context, thin, vaguely divided in

a rather poorly gelatinized subpellis and a thin, discontinuous and disrupted suprapellis of intricate, small-celled and highly branched hyphal endings; these very thin-walled and also with thin septa; terminal cells very small and short, measuring (8–)12.5–**17**–21.5(–27) × (2.5–)3.5–**4.1**–4.5(–5) µm, mostly very irregular and often nodulose in form, with local contortions and inflations, not attenuated but rounded-obtuse or somewhat inflated near the tips, in the centre of pileus similar, somewhat more regular and with terminal cells of similar size, (8.5–)11.5–**14.7**–18(–20) × (2.5–)3–**3.6**–4.5(–5) µm. Pileocystidia distinct, dispersed, 1–3 celled, of very variable length, mostly situated at the cap surface and with terminal cells 12–**17.5**–23.5 × 5.5–**6.6**–8 µm, ellipsoid, fusiform or rarely clavate, obtuse, but some also longer and cylindrical, ascending from the subpellis, absent in the underlying trama; contents granular to crystalline, refringent, hardly reacting with sulfovanillin. CLAMP CONNECTIONS absent in all parts.

COMMENTARY — The only mycologist who published on the type of *R. sericella* was Hesler (1960), unfortunately without any comment on the quite distinctive pileipellis. Singer also examined the type as evidenced by the note he left with the type specimen stating that "*R. sericella* = *R. obscuriformis* Murrill = *R. xerampelina*". We disagree completely with the proposed synonymy, these three taxa being very different as shown here.

Using the synoptic key of Kibby & Fatto (1990) the combination of A?KNPT (with the question mark allowing for variation in our interpretation of the second feature: the "readily" peeling cap) leads either to *R. rosea* Quél. (choosing I = ⅓–¾ peeling) or to *R. sericeonitens* Kauffman (choosing J = ¾ to completely peeling), the latter being indeed the species that is suggested by Murrill himself as being very close to *R. sericella.* Both species are quite different from *R. sericella.*

Use of European *Russula*-keys results in similar placements as for some of the species discussed above and leads to the mild or faintly acrid species around *R. krombholzii* Shaffer in *R.* subgen. *Russula* when using Romagnesi's key (1985) or to a placement in *R.* subgen. *Tenellula* Romagn. with Bon's key (2002).

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The authors would like to thank the curators of the mycological herbaria of FLAS (Gainesville, Florida) and TENN (Knoxville, Tennessee) for their efficiency and R.H. Petersen at TENN for access to Hesler's personal notes and duplicate specimens. A study visit to the Paris' Herbarium by Adamčík was funded through the Synthesys program of the European Union (FR-TAF-3122 and FR-TAF-5110), his further studies funded national project Vega 2/0028/11. Overseas travel of the senior author has been funded by the research program "État et structure phylogénétique de la biodiversité actuelle et fossile" (Dir. Ph. Janvier) at the Paris' Museum. Reviewers of the manuscript, André Fraiture and Pierre-Arthur Moreau, are acknowledged for valuable comments.

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New species and new records of *Crepidotus* from the northwest region of São Paulo State, Brazil

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ABSTRACT The study of *Agaricales* collections gathered in the northwest region of São Paulo State, Brazil revealed the occurrence of four species of *Crepidotus*. Two of them, *C. flavus* and *C. longicystidiatus*, are proposed as new, while *C. apodus* and *C. defibulatus* are recorded for the first time in the northwest region of São Paulo State.

KEY WORDS diversity, South America, taxonomy

Introduction

The genus *Crepidotus* (Fr.) Staude includes members with a lignicolous habit, pleurotoid basidiomata often bearing a reduced stipe, and pilei that are glabrous to densely villose especially near the rear portion. The spore print is in shades of pale brown or sometimes yellowish, and spores that may be smooth or ornamented but which always lack a germ pore. Pleurocystidia are normally absent, but cheilocystidia are mostly present. Hyphae may be clamped or not.

Spore ornamentation and the presence or absence of clamp connections are the basis of infrageneric classification in *Crepidotus* by Singer (1947, 1973) and Hesler & Smith (1965). In her study of European species, Senn-Irlet (1995) based her infrageneric classification on pileus trama type, including the presence or absence of a gelatinous layer.

In Brazil, *Crepidotus* is represented by 36 taxa, of which 10 — *C. apodus*, *C. applanatus* var. *subglobiger*, *C. candidus*, *C. catamarcae*, *C. crocophyllus*, *C. defibulatus*, *C. martinii*, *C. palmarum*, *C. polylepidis*, *C. quitensis*, *C. rubriceps*, *C. scymnodes*, *C. stromaticus*, *C. tigrensis*, *C. uber*, *C. variabilis* — occur in São Paulo State (Capelari 2007). Paraná and Rio Grande do Sul are the states with the next highest numbers of known species (Senn-Irlet & Meijer 1998, Rick 1930, 1938, 1961). Singer (1989) cited two species for Amazonas (*C. igapoensis*,

C. pilosiceps), which are known only by the types. Singer (1973) described *C. guzmanii* and mentioned *C. truncatus* for Pernambuco State. For the state of Rio de Janeiro, Singer (1973) cited *C. albidus* var. *bisporus* and both he and Bandala et al. (2006) mentioned *C. septicoides*.

This paper, which deals with collections from forest fragments in the northwest region of São Paulo State, proposes two new species: *C. longicystidiatus* and *C. flavus*.

Materials & methods

Materials were collected in four private forest remnants within the northwest region of São Paulo State. These remnants are composed of semideciduous seasonal forest, which is very fragmented and surrounded by either sugar cane or orange plantations or pasture. The human impact on these places probably will not be reverted and they tend to disappear.

For microscopic analyses, dried material was rehydrated in 70% ethanol followed by 5% KOH. For spore and cheilocystidia dimensions, 20 measurements were taken for each specimen; Qm represents the mean length/width quotient of the total spores measured. All microscopic illustrations were made with the aid of a drawing tube. The colors of fresh material, when possible, were compared with those described by Küppers (1979), and the specimens were deposited at the herbarium of the Instituto de Botânica (SP).

Taxonomy—new species

Crepidotus flavus Capelari, **sp. nov.** FIGS 1A, 2

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Pileus 3 17 mm latus, flabellatus, flavus vel ochraceus, brunneus prope substrato, pruinosus, margine flava vel ochracea, involuta. Lamellae adnatae, flavae vel ochraceae. Stipes nullus. Basidiosporae 8.7 × 8.7 µm, globosae, valde verruculosae. Pleurocystidia nulla. Cheilocystidia 19.3 29.2 × (5.2) 7.0 9.2 µm, numerosa, flageliformia, leavia, interdum cum apicibus incrustatis. Trama pileorum flavida, hyphae fibulatae, 6.2 12.5 µm latae. Trama lamellarum regularis, flavida, hyphae fibulatae, 6.2 8.7 µm latae. Pileipellis ex hyphis repentibus vel erectiusculis, fibulatis. Gregarius, supra trunco caduco.

TYPE: BRAZIL, SP, Matão, (21°37'14"S 48°32'14"W), 12 December 2007, F. Karstedt 1011 (**holotypus** in herbarium SP asservatur).

ETYMOLOGY: Latin, yellow, the basidiomata color.

MACROCHARACTERS: PILEUS 3–17 mm broad, flabelliform, yellow to ochraceous, brown near the attachment to the substrate, margin yellow to ochraceous yellow, pruinose at the rear portion, dry, not hygrophanous, margin plane to slightly incurved, smooth. LAMELLAE adnate, diverging from a common central point, distant, with 1–2 series of lamellulae, yellow to ochraceous. STIPE absent. CONTEXT yellowish, thin.

MICROCHARACTERS: BASIDIOSPORES 8.7 × 8.7 µm, Q_m = 1.0, uniformly globose, ornamented, strongly verrucose, light brown, thick-walled, inamyloid. BASIDIA

Fig. 1. A. *Crepidotus flavus* (type); B. *Crepidotus longicystidiatus* (type). Bars = 1 cm.

not seen. PLEUROCYSTIDIA absent. CHEILOCYSTIDIA 19.3–29.2 × (5.2–)7.0–9.2 µm, numerous, turning the margin completely sterile, ventricose, smooth, hyaline, thin-walled, some with apical encrustations, clamp connections at the base not seen. PILEUS TRAMA thin, yellowish, with cylindrical, thin-walled hyphae, 6.2–12.5 µm diam, hyaline, clamped, ungelatinized. HYMENOPHORAL TRAMA regular, yellowish, with cylindrical, slightly thin-walled hyphae, 6.2–8.7 µm diam, hyaline, clamped, ungelatinized. PILEIPELLIS light brown, a cutis of prostate to some loose and suberect yellowish to light brownish hyphae, 3.7 µm diam, thick-walled, clamped.

HABIT AND HABITAT: Gregarious, on fallen trunk.

COMMENTS: Macroscopically, this material fits very well with the illustration of *Crepidotus stromaticus* (Cooke & Masse) Sacc. published by Hemmes & Desjardin (2002). According to these authors, *C. stromaticus* from Hawaii had been previously referred to *C. citrinus* Petch and *C. sulphurinus* Imazeki & Toki. Literature on *C. stromaticus* is limited, although it was cited for Brazil by Rick (1961) in material that needs revision.

Pilát (1950) described the Australian type material of *C. stromaticus* as "*albido-isabellino . . . Pileus 10–15 mm. latus . . . Sporae . . . distincte dense obtuse verrucosae, 8–8.5 × 6.7–7.5 µ*", and considered it identical to *C. pogonatus* (Kalchbr.) Sacc. However, in the same paper, he described the basidiospores of *C. pogonatus* as "*punctato-verrucosae . . . 5–5.6 µ diam.*," much smaller than he measured for *C. stromaticus*. On the other hand, Singer (1955) described the type of *C. stromaticus* as "strongly punctate and somewhat rough, about 7–7.5 µm in diameter" and regarded *C. stromaticus* so close to *C. nephrodes* that a specific separation could hardly be justified. Information by Ripková et al. (2005) and Bandala et al. (2008) suggests that *C. nephrodes* is a different taxon and should even be considered a synonym of *C. crocophyllus*, a broadly distributed temperate fungus with variable pigmentation that was already mentioned for São Paulo State by Rick (1930, 1961).

Crepidotus sulphurinus (Imazeki & Toki 1954), according to the protologue, has bisporic basidia and basidiospores that are 7–10 × 6–9 µm, subglobose, apiculate, brownish-yellow, and echinulate-verrucose. *Crepidotus flavus,* on the other hand, produces truly globose, perhaps less ornamented basidiospores (as far as I know there are no available MEV photographs of *C. sulphurinus*), and ventricose cheilocystidia (some apically encrusted) and not clavate as illustrated by Imazeki & Toki (1954).

Singer (1953) identified a yellow *Crepidotus* from Argentina as *C. citrinus* growing together on the same trunk with another species he identified as *C. fulvifibrillosus* Murrill, which Ripková et al. (2005) consider a synonym of *C. crocophyllus*. *Crepidotus citrinus* was described from Sri Lanka and, according

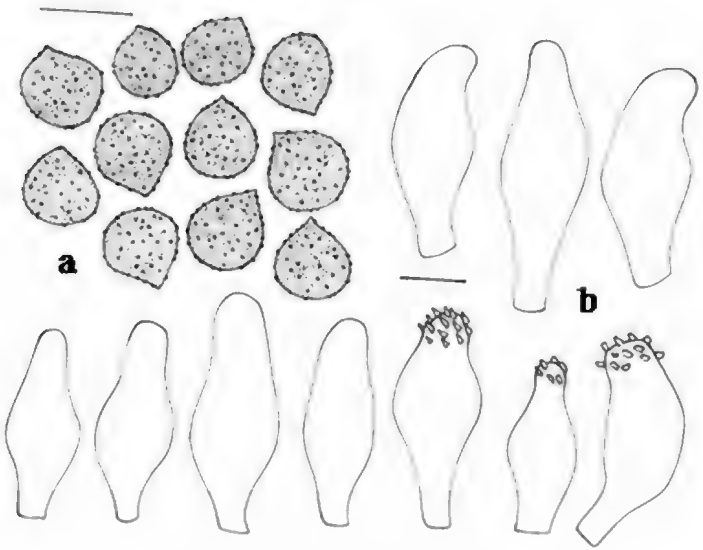

Fig. 2. *Crepidotus flavus* (type):
a. basidiospores; b. cheilocystidia. Bars = 10 µm.

to Singer (1955), the type has globose basidiospores that are 7–8 × 7–8 µm, with strongly imbedded spinules that project slightly beyond the episporium so that the spore surface appears rough in immersion oil. Singer (1955), however, did not mention cheilocystidia size and shape. The Argentinean material of the same species described by Singer (1953) has globose basidiospores that are 6.8–8.3 µm, 2-, 3-, or 4-spored basidia, and hyaline to light yellow versiform cheilocystidia, the majority of which have irregular excrescences but lack encrustations and which measure (15.0–)27.5–42 × (4.0–)6.8–11(–14.5) µm. No other modern description of *C. citrinus* was found in order to clarify the cheilocystidia. Indeed, *C. citrinus* sensu Singer appears to be the closest species to the new Brazilian one, but they differ in cheilocystidia shape and size.

Horak (1978) described two species with yellow colors and encrusted cheilocystidia—*C. aureus* from New Caledonia and *C. parietalis* from New Zealand—that can be compared with *C. flavus*. *Crepidotus flavus* differs from *C. aureus* in basidioma dimensions, less ornamented pileus surface, and basidiospore and cheilocystidial morphology, with *C. aureus* producing smaller (6–7 µm), subglobose spores and cheilocystidia that are fusoid to lageniform, basally thin-walled and apically thick-walled (≤ 1.5 µm). From *C. parietalis*, *C. flavus* differs in pileus color (paler in *C. parietalis*) and especially by the basidiospore dimension and shape (5–6.5 µm and globose in *C. parietalis*) and the absence of thick-walled hyphae at the pileipellis.

Crepidotus longicystidiatus Capelari, **sp. nov.** FIGS 1B, 3

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Pileus 3–7 mm latus, primum flabellatus, deinde convexus vel plano-convexus, dorsaliter affixus in substrato tum circularis vel reniformis, subroseus, pilosus; margine plana ad involutam. Lamellae adnatae, vinaceo-roseae. Stipes nullus. Basidiosporae (6.2–)7.5(–8.7) × 6.2–7.5 µm, globosae vel subglobosae, verruculosae. Basidia 20–25 × 5.0–6.0 µm, clavata, tetraspora. Pleurocystidia nulla. Cheilocystidia 60–116 × 5.0–7.0 µm (14.0 µm apicem), cylindracea ad flexuosa, hyalina, cum fibulis in base. Trama lamellarum regularis, hyphae fibulatae, 6.2–11.2 µm latae. Pileipellis ex hyphis repentibus composite, fibulatae, 6.2–10.0 µm latis. Gregarius ad ramo putridos.

TYPE: BRAZIL, SP, Turmalina, (20°00'13"S 50°26'02"W), 27 November 2007, Capelari 4358 (**holotypus** in herbarium SP asservatur).

ETYMOLOGY: Latin, because of the very long cheilocystidia.

MACROCHARACTERS: PILEUS 3–7 mm broad, flabelliform in primordial stages, becoming convex or plane-convex, dorsally attached to substrate, then circular, subcircular or more or less reniform when seen from hymenophore, light pinkish, dry, not hygrophanous, surface pilose, margin plane to incurved, smooth. LAMELLAE adnate to a common central or excentric point, distant, wit 1–2 series of lamellulae, vinaceous pink (near $N_{50}A_{60}M_{70}$), edges lighter. STIPE absent. CONTEXT thin.

MICROCHARACTERS: BASIDIOSPORES (6.2–)5.0–7.0(–8.7) × 6.2–7.5 µm, Q_m = 1.19, globose to subglobose, ornamented, verrucose, ferruginous-brown, slightly thick-walled, inamyloid. BASIDIA 20–25 × 5.0–6.0 µm, clavate, four-spored, hyaline, thin-walled. BASIDIOLES numerous. PLEUROCYSTIDIA absent. CHEILOCYSTIDIA 60–116 ×5.0–7.0 µm, up to 14.0 µm at the apex), numerous, cylindrical, narrowly clavate, flexuous, smooth, hyaline, slightly thin-walled, clamped at the base. PILEUS TRAMA thin, of cylindric, slightly thin-walled hyphae, 6.2–10 µm diam, hyaline, clamped, ungelatinized. HYMENOPHORAL TRAMA regular, of cylindric, slightly thin-walled hyphae, 6.2–11.2 µm diam, hyaline, clamped, constricted at the septum, ungelatinized. PILEIPELLIS a cutis of undifferentiated flexuous hyphae, 6.2–10 µm diam, hyaline to slightly brownish, slightly thick-walled, clamped, with small clamp connections. PILEUS HYPHAE at the substrate hyaline slightly brownish hyphae, 2.5–3.7 µm diam, moderately thick-walled, sometimes encrusted, branched, clamped.

HABIT AND HABITAT: Gregarious, on fallen branch.

COMMENTS: Of the species with pink or pinkish pilei or lamellae, the new species can be compared with *C. roseus* Singer, *C. roseolus* Singer, and *C. velutinoaffinis* Singer, described in Singer's 1973 monograph of *Crepidotus* for the Neotropics.

Crepidotus roseus differs in basidiospore morphology and cheilocystidia size and is characterized by less broad (6.5–7.0 × 5.3–5.8 µm) subglobose basidiospores and smaller (30–48 × 7.5–13 µm) cheilocystidia. The new

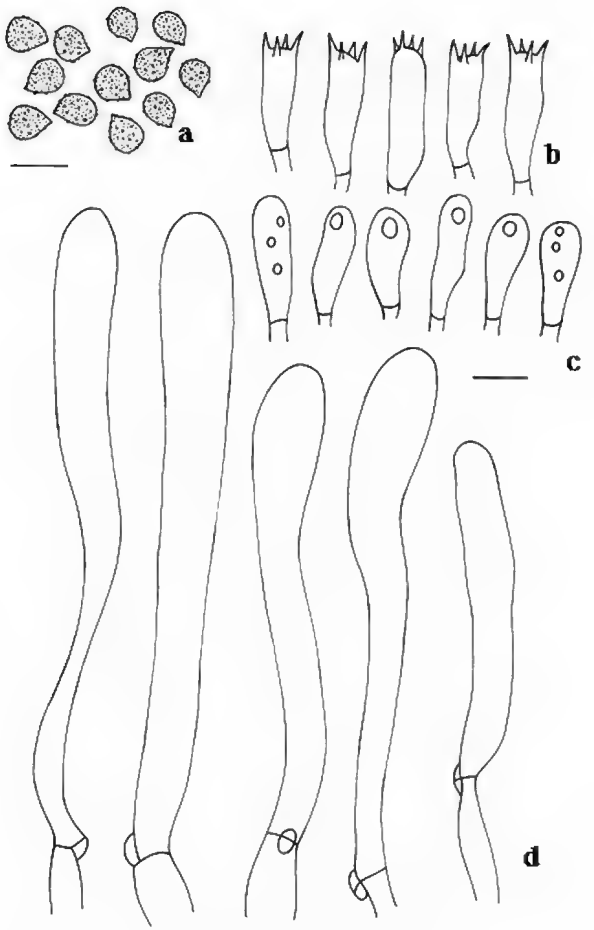

Fig. 3. *Crepidotus longicystidiatus* (type):
a. basidiospores; b. basidia; c. basidioles; d. cheilocystidia. Bars = 10 µm.

species differs similarly from *C. roseolus*, which has smaller (5.0–6.2 µm, most ~5.5 µm) punctate globose basidiospores and cheilocystidia that are 32–35 × 6.7–9.3 µm, ventricose in the lower and upper portions and slightly constricted in the middle, and often with one or two finger-like appendages. *Crepidotus velutinoaffinis* differs in its small (2 × 1 mm) stipe (lacking in *C. longicystidiatus*) and cheilocystidia, which are 40–61 × 3.8–7 µm, cylindric or cylindric-subclavate to slightly ventricose with apices that are mostly subcapitate or at least slightly constricted just below the apex or occasionally equal and rounded or attenuate (rarely acute).

Crepidotus rubrovinosus Bandala et al. (2006), with a reddish or red-wine pileus described from Mexico, differs in its persistently reddish fimbriate lamellar edges, oblong/ellipsoid basidiospores with a suprahilar depression and ornamentation comprising short, broad and sinuous blunt bulges and ridges that often anastomose to form a subreticulate pattern, and cheilocystidia that are cylindric-clavate to clavate, narrowly utriform, 35–138(–147) × 3–7(–8) µm, occasionally septate, sinuous toward the base, apex rounded or subcapitate, rarely attenuated, and, at times, bifurcate or lobate (Bandala et al. 2006).

Taxonomy—new records

Crepidotus apodus Capelari, Hoehnea 34: 76. 2007.

MATERIAL EXAMINED: BRAZIL, SP, Onda Verde, 20°2'08"S 48°49'35"W, 09 December 2008, Capelari 4426 (SP).

COMMENTS: *Crepidotus apodus* produces many very small yellowish basidiomata that colonize shrubs on the forest floor. It was described from Parque Estadual das Fontes do Ipiranga (south of São Paulo city), and since its publication, it has frequently been collected in other protected areas of São Paulo State. Description and illustrations can be found in Capelari (2007).

Crepidotus defibulatus Singer, in Singer & Digilio, Lilloa 25: 410. 1952 ["1951"].

MATERIAL EXAMINED: BRAZIL, SP, Turmalina, 20°00'13"S 50°26'02"W, 27 November 2007, Capelari 4348 (SP).

COMMENTS: *Crepidotus defibulatus* is one of the few *Crepidotus* species with ornamented basidiospores and clampless hyphae. It occurs in Argentina (Singer 1973, type locality) and São Paulo State, Brazil (Capelari 2007). Descriptions can be found in Singer & Digilio (1952), Singer (1973), and Capelari (2007, with illustrations).

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Pilidiella crousii sp. nov. from the northern Western Ghats, India

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Abstract The coelomycete genus *Pilidiella* (*Schizoparmaceae, Diaporthales*) was recently revised to include species with hyaline to pale brown conidia in contrast to the dark brown conidia of *Coniella*. The present paper describes a new species of *Pilidiella, P. crousii*, based on a molecular phylogenetic analysis of the ITS nrDNA and its unique conidial morphology. *Pilidiella crousii* is associated with severe fruit drop of *Terminalia chebula* (*Combretaceae*) in natural forests of Mahabaleshwar in the Western Ghats of India.

Key words anamorphic fungi, plant pathogen, *Schizoparme*

Introduction

The exploration of microfungal diversity of the Western Ghats, which is one of the biodiversity hot spots of the world, is the prime goal of the National Fungal Culture Collection of India (NFCCI). The NFCCI aims to explore fungi from this region for their biotechnological and bioprospecting potential (Rajeshkumar et al. 2010; Singh et al. 2009). During July 2010 a routine survey was conducted to explore the microfungal diversity in the natural forests of Mahabaleshwar, situated in the northern part of the Western Ghats, India, at 17°58'N and 73°43'E. Discovered during this survey, an interesting species of *Pilidiella* Petr. & Syd. was found on fallen fruits of *Terminalia chebula,* associated with severe fruit infection followed by fruit drop. The aim of the present study is to identify the species of *Pilidiella* associated with fruit drop of *Terminalia* in India.

Materials & methods

Isolates and morphology Conidiomata of the fungus were directly isolated from the surface of fallen fruits and observed under a Nikon Binocular stereo microscope

(Model SMZ – 1500 with Digi-CAM, Japan). Single conidial cultures were established on 2% Potato Dextrose Agar plates (PDA; Crous et al. 2009). For morphotaxonomic studies and photomicrographs an Olympus CX-41 (Japan) microscope was used. Conidia and conidiomata were mounted in lactic acid cotton blue and measured using an ocular micrometer, with 30 observations per structure. Colony characteristics in culture were studied on different media, viz. 2% Malt Extract Agar (MEA), Potato Carrot Agar (PCA), and PDA (Crous et al. 2009). Herbarium specimens were deposited in Ajrekar Mycological Herbarium (AMH); the culture was accessioned and preserved in the National Fungal Culture Collection of India (WDCM-932), Agharkar Research Institute, Pune, India.

Polymerase chain reaction and sequencing Total DNA was extracted from cultures grown on PDA plates for two weeks at 25 °C, using a DNA extraction kit as per the manufacturer's instructions (MP Biomedicals GmbH, Germany). Fragments containing the region encoding the ITS 1-5.8S-ITS 2 were amplified using primer pairs ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS5 (5'-GGA AGT AAA AGT CGT- AAC AAG G-3') (White et al. 1990). PCR was performed in a 25 µl reaction using 2 µl of template DNA (10 ng - 25 ng), 0.5 U of Taq DNA polymerase (Genei, Bangalore, India), 2.5 µl of 10X Taq DNA polymerase buffer, 0.5 µl of 200 µM of each dNTPs (Genei, Bangalore, India), 0.5 µl of 10 pmol primer, H_2O (Sterile Ultra Pure Water, Sigma) qsp 25 µl. Amplification was performed on an Eppendorf Mastercycler AG using the following parameters: 5 min step at 95 °C, followed by 30 cycles of 1 min at 95 °C, 30s at 56 °C and 1 min at 72 °C for ITS region amplification, then a final 7 min extension step at 72 °C. The PCR products were purified with Axygen PCR cleanup kit (Axygen Scientific Inc, CA, USA) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sequencing reactions were run on an ABI 3100 automated DNA sequencer (Applied Biosystems, USA).

Sequence alignment and phylogenetic analysis Sequence alignment of partial ITS1-5.8S-ITS2 of *P. crousii* was performed manually using the text editor option of the software Molecular Evolutionary Genetics Analysis (MEGA) software v4.0. (Tamura et al. 2007). The manually edited sequences of NFCCI 2213 were deposited in the NCBI sequence nucleotide database (HQ264189). They were also subjected to a BLAST search. The partial ITS sequences were aligned using Clustal W together with the homologous regions of ITS of closely related species of *Pilidiella* and *Schizoparme* Shear. For ITS, the matrix was analyzed with the Maximum Parsimony method using the Tamura model (Tamura et al. 2007) to calculate the sequence divergence, and the bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option).

Results

DNA phylogeny For the phylogenetic analysis of the genus *Pilidiella*, the alignment of 557 bp of the ITS sequence data included 450 positions in the final data set. The analysis of ITS1–5.8S–ITS2 sequence presented here (Fig. 1) reveals a significant association of *Schizoparme* and its anamorphic genus *Pilidiella*. The phylogenetic analysis based on ITS sequences resulted in four

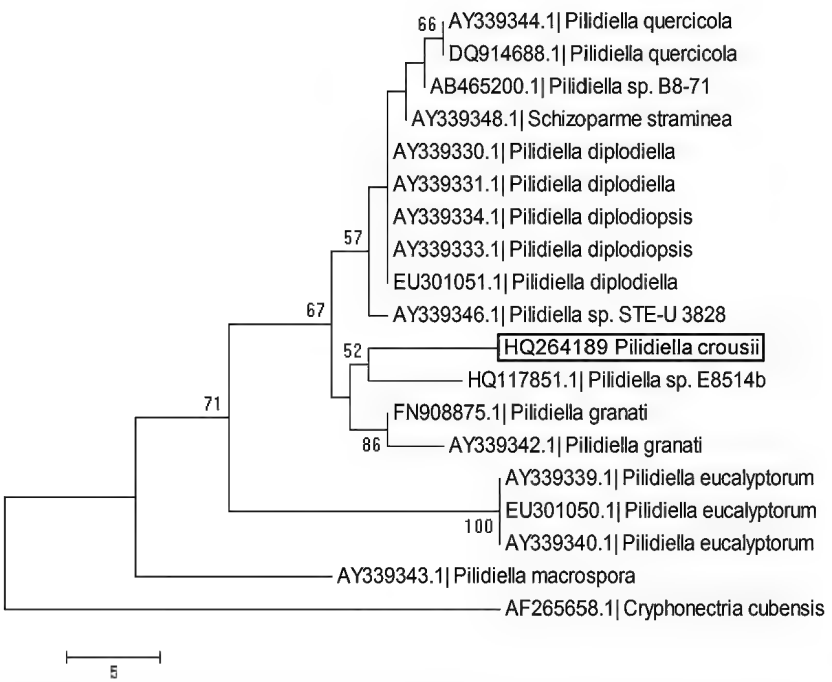

FIG. 1. Phylogenetic tree based on aligned internal transcribed spacer sequences of *Schizoparme* and its anamorphic species of *Pilidiella*. The consistency index is (0.660000), the retention index is (0.821053), and the composite index is 0.674127 for all sites and parsimony-informative sites. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The MP tree was obtained using the Close-Neighbour-Interchange algorithm with search level 3 in which the initial trees were obtained with the random addition of sequences (10 replicates). The tree is drawn to scale; with branch lengths calculated using the average pathway method and are in the units of the number of changes over the whole sequence.

major clades, *P. diplodiella* (Speg.) Crous & Van Niekerk, *P. diplodiopsis* Crous & Van Niekerk, *P. quercicola* (Oudem.) Petr. and *S. straminea* Shear clustered together and formed a major group. *Pilidiella eucalyptorum* Crous & M.J. Wingf. formed a unique clade (100% similarity). *P. macrospora* (71% similarity) clustered as a unique clade. The new species, *P. crousii,* clustered with *Pilidiella* sp. (HQ 117851.1) (52% similarity), which was sister to *P. granati* (Sacc.) Aa. *Cryphonectria cubensis* (Bruner) Hodges was chosen as outgroup, as it belongs to the *Cryphonectria–Endothelia* complex that is allied to the *Schizoparme* complex (Castlebury et al. 2002).

Taxonomy — Based on a DNA comparison with other species currently in GenBank (Fig. 1), and its unique morphological characters (Fig. 2), such as the highly variable conidial shape, size and length-width ratio, the species of *Pilidiella* from *Terminalia* can be distinguished from the other taxa in this genus, and hence is described as new to science.

Pilidiella crousii Rajeshkumar, S.K. Singh & Hepat, **sp. nov.** Fig. 2

MycoBank MB 518897

In fructis. Pycnidia globosa vel subglobosa, 124–280 × 115–190µm, laeves, pariete multitunico ex 2–4 stratis et textura angulari. Conidiophora 15–25 × 2.5–3 µm, simplicia vel ramose. Cellulae conidiogenae 6–12 × 2–3µm, simplices, laeves. Conidia primo hyalinia, deinde pallida vel modice brunnea, forma et amplitudine valde variabilibus, laevia, ellipsoidea vel anguste ellipsoidea, apice acute rotundato vel subobtuso, basi truncata, (6–)7–12(–13.5) × (2.5–)3–5 µm (plus minusve 9.5 × 4.0 µm), ratione longitudinis/latitudinis 2.2–2.3.

Holotype: India, Mahabaleshwar, Western Ghats, Maharashtra, on fallen fruits of *Terminalia chebula* (*Combretaceae*) July 2010, K.C. Rajeshkumar (AMH 9406; ex-type culture NFCCI 2213.)

Etymology: '*crousii*' named in honour of Prof. dr. Pedro W. Crous, Director, Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands, for his scientific contribution to this fungal group.

On fruits, forming pustule-like structures erupting through pericarp, scattered over fruit surface; conidiomata aggregated in a dark reddish brown mass. Conidiomata pycnidial, globose to sub-globose, 124–280 × 115–190 µm, smooth; wall of textura angularis, initially hyaline, becoming pale to medium brown, consisting of 2–4 layers. Conidiophores developing from a basal or central, hyaline cushion of cells, simple or branched, densely packed, encased in mucous, 15–25 × 2.5–3 µm. Conidiogenous cells simple, smooth, 6–12 × 2–3µm. Conidia initially hyaline, becoming pale to medium brown when mature, smooth, highly variable in shape and size, straight, occasionally slightly curved, ellipsoidal to narrowly ellipsoidal, with subobtuse apex and base or truncate base in a few conidia, but acutely rounded apices also present in others, bi- to multiguttulate, (6–)7–12(–13.5) × (2.5–)3–5 µm (mean 9.5 × 4.0 µm), length-width ratio = 2.2–2.3.

Teleomorph: Unknown; no sexual state or fungus resembling *Schizoparme* was present on any specimen examined.

Colonies on PDA fast growing, 79 mm diam after 7 days and 90 mm diam after 10 days, white initially, later turning pale brown to dark brown, floccose, reverse pale to dark brown. Colonies on MEA fast growing, 80 mm diam after 7 days, initially white, brownish when mature, margin irregular, reverse dark gray-brown. Colonies on PCA fast growing, 60 mm diam after 7 days, pale brown margin regular, colonies reverse dark brown.

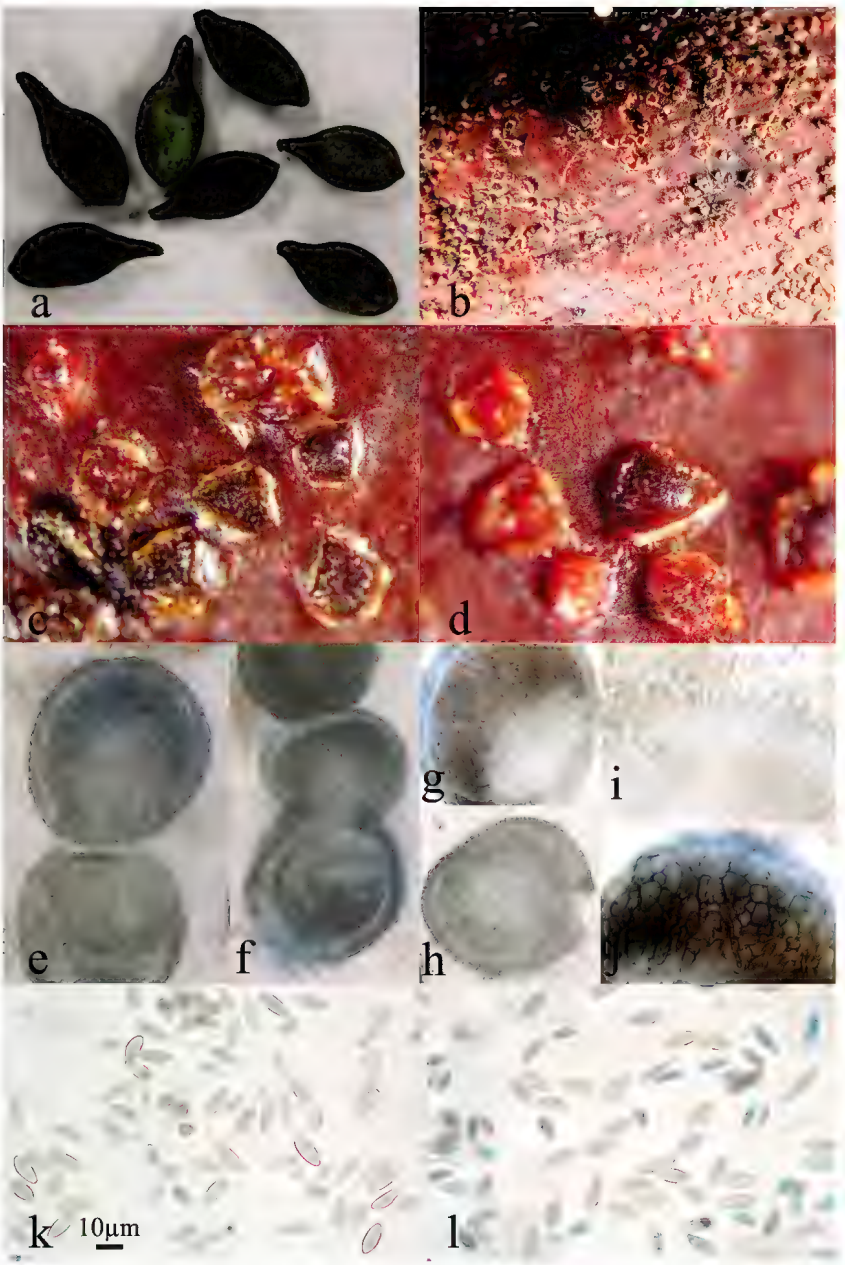

FIG. 2. *Pilidiella crousii* (holotype). a. Fallen fruits of *Terminalia chebula*. b–d. Conidiomata on fruits. e–h. Pycnidia in culture. i. Conidiophores developing from basal pad of tissue. j. Pycnidial wall. k–l. Variable shaped conidia. Bars: k, l = 10 µm.

Discussion

Petrak & Sydow (1927) established the genus *Pilidiella* with hyaline to pale brown conidia, differentiating it from *Coniella* Höhn., which has dark brown conidia. Later, von Arx (1957, 1972) also supported the differentiation of these genera based on their conidial pigmentation. However, Sutton (1980) and Nag Raj (1993) treated *Pilidiella* as a synonym of *Coniella*.

Castlebury et al. (2002) determined that *Pilidiella* with its teleomorph *Schizoparme* formed a distinct lineage within the *Diaporthales*, representing a genus separate from *Coniella*. Later, Van Niekerk et al. (2004) conducted an extensive study of the group, including a thorough molecular analysis based on ITS, EF1-α and LSU gene sequence data. Based on all three data sets, they confirmed the separation of *Pilidiella* (typified by *P. quercicola* (Oudem.) Petr. [= *P. castaneicola*]) from *Coniella* (typified by *C. pulchella* Höhn. [= *C. fragariae*]). *Pilidiella* is characterized by having species with hyaline to pale brown conidia with a length-width ratio >1.5 in contrast to dark brown conidia of *Coniella* with length-width ratio ≤1.5.

Samuels et al. (1993) linked several species of *Coniella* to species of *Schizoparme*, which they regarded as a member of *Diaporthales* (*Melanconidaceae*). However, Castlebury et al. (2002) suggested that the *Schizoparme*-complex is representative of an undescribed family in the *Diaporthales*, with *Coniella* and *Schizoparme–Pilidiella* as separate genera. In a further study, Rossman et al. (2007) established *Schizoparmaceae* Rossman (type genus: *Schizoparme*) to accommodate these genera, with diagnostic characteristics "*Ascomata fusca vel nigra, collapsa, erumpentia, superficialentia. Asci annulo apicali distincto praediti, ad maturitatem separati, paraphyses nullae. Ascosporae non septatae*". The pycnidial anamorphs of this family are *Coniella* and *Pilidiella*. The type species of *Schizoparme* is *S. straminea*, which is linked to the anamorph *Pilidiella castaneicola* (Ellis & Everh.) Arx. In contrast with the *Schizoparme* teleomorphs associated with *Pilidiella*, no teleomorph has thus far been reported for *Coniella* (Rossman et al. 2007).

In India, Sharma et al. (1985) studied the genus *Coniella* from *Eucalyptus* plantations in Kerala State. They reported *C. castaneicola* (Ellis & Everh.) B. Sutton and *C. fragariae* (Oudem.) B. Sutton causing a leaf spot disease in *E. grandis* and *E. tereticornis*. Van Niekerk et al. (2004) pointed out that the isolates from *Eucalyptus* treated as *C. fragariae* should be recognized as *P. eucalyptorum*. *Coniella granati* (Sacc.) Petr. & Syd. was reported as a saprobe on leaf litter of *Tectona grandis* (Mary & Sankaran 1991; Sankaran 1994). Rajeshkumar (2007) explored the pathogenic microfungal diversity in natural forests in the Kerala part of the Western Ghats, and reported four species of *Coniella*: *C. australiensis* Petr. on *Macaranga peltata*, *C. minima* B. Sutton & Thaung on *Garcinia gummi-gutta*, *C. petrakii* B. Sutton on *Careya arborea* and *Syzygium caryophyllatum*, and *C. fragariae* on more than 10 different hosts plants in natural forests.

Coniella spp. have been reported as important foliar pathogens of *Terminalia* spp. in India. *Coniella terminaliae* (Firdousi et al. 1994) was identified as a new species causing necrotic spots on *Terminalia tomentosa* (IMI 823384) from the forests of Gopalpura (Sagar), Madhya Pradesh. This species is close to *C. fragariae* in shape and colour of its pycnidia and conidia, but its conidia are smaller (2—8 × 3—5 µm) and globose to subglobose in shape. Rajeshkumar (2007) also recorded foliar diseases caused by *Coniella fragariae* on *Terminalia chebula* and *T. paniculata* in natural forests of Kerala. *Coniella macrospora* (van der Aa 1983) is the only species under this genus so far recorded on *Terminalia ivorensis* from the Ivory Coast, but its conidia are much larger, (18.3–)25–29 (–32.5) × (13–)16–20(–21.5) µm.

In the present study, *Pilidiella crousii* is proposed as a new species based on the ITS sequence analysis (Fig. 1) and highly variable shape and size of its conidia (Fig. 2). The morphological characteristics of *P. granati* differ from those of the new species. In *P. granati* the conidia are ellipsoidal, 9–16 µm long, and length- width ratio is 1.9. In *P. crousii*, the conidia are ellipsoidal or narrowly ellipsoidal, (6–)7–12(–13.5) µm long, with a length -width ratio of 2.2–2.3. The conidial length-width ratio of *P. crousii* is close to that of *P. diplodiella,* but the conidia in *P. diplodiella* are longer. Also the ITS sequence analysis clearly differentiates these two species.

Appendix

In their treatment of *Pilidiella* and *Coniella*, Van Niekerk et al. (2004) referred to *Coniella macrospora* as "*Pilidiella macrospora*", but did not make a valid combination. This combination is proposed here:

Pilidiella macrospora (Aa) Crous & Van Niekerk, comb. nov.

MycoBank MB 517391

Basionym: *Coniella macrospora* Aa, Proc. Kon. Ned. Akad. Wetensch., C 86: 121. 1983.

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Calocera bambusicola sp. nov. and *C. sinensis* newly recorded from Taiwan

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Abstract — *Calocera bambusicola* is presented as a new species, based on specimens collected from rotten culms of bamboo (*Pseudosasa usawai*) near a sulfur hot spring on Yangminshan mountain in Yangminshan National Park, Taipei, Taiwan. This new species morphologically resembles *C. sinensis* but differs in its much smaller basidiocarps, narrower basidiospores, much narrower contextual hyphae, and growth on bamboo culms. ITS differences also separate these two species. A key to species of *Calocera* with nodose-septate hyphae is provided, and *C. sinensis* is newly reported from Taiwan.

Key words — *Dacrymycetaceae*, *Dacrymycetales*, taxonomy

Introduction

The genus *Calocera* (Fr.) Fr. (*Dacrymycetaceae* J. Schröt., *Dacrymycetales* Henn.) is ecologically saprobic, causing a brown rot in wood. McNabb (1965) treated twelve species and infraspecific varieties in his worldwide monograph of *Calocera*, among which only four species possess clamped hyphae. Reid (1974), who treated five *Calocera* species in his monographic survey of British *Dacrymycetales*, included a new species, *C. pallidospathulata* D.A. Reid. Peng et al. (1992) recognized six species of *Calocera* from China, including the newly described species *C. mangshanensis* B. Liu & L. Fan (Liu & Fan 1989) and *C. morchelloides* B. Liu & L. Fan (Liu & Fan 1990). The three species published after McNabb's (1965) monographic work all have simple-septate hyphae. Previous taxonomic study of *Calocera* was based on morphological analyses. DNA sequence analyses have never been used to test species delimitations. So far, only *Calocera cornea* (Batsch) Fr. and *C. viscosa* (Pers.) Fr. sequences have been submitted to the GenBank database (Shirouzu et al. 2009).

In 1999-2001, several specimens of *Calocera* were collected from rotten culms of the bamboo species *Pseudosasa usawai* near a sulfur spring on Yangminshan

mountain in Taipei, Taiwan. Basidiocarps of these *Calocera* specimens are very small and bear nodose-septate hyphae. Morphological and ITS sequence analyses reveal that these specimens represented an undescribed species, *C. bambusicola,* which is described here. *Calocera sinensis,* which resembles the new species, is here reported for the first time from Taiwan.

Materials & methods

Morphology — Specimens for this study were collected in Taiwan during 1992–2007 and are deposited at the herbarium of the National Museum of Natural Science of ROC (TNM). For microscopic observations, the sections were stained with 1 % aqueous Phloxine in 5 % KOH. Melzer's reagent (IKI) was employed to detect amyloidity and dextrinoidity.

Phylogeny — DNA was extracted from dried fruiting body tissue or cultured mycelia as described in Wu et al. (2007) from eight strains representing six species (Table 1). Both DNA strands were sequenced by ITS1 and ITS4 primers (White et al. 1990). Consensus data from the forward and reverse sequences were matched using BioEdit 7.0.4.1 (Hall 1999). Alignment was performed using Clustal X 1.83 (Thompson et al. 1997) and adjusted by hand also using BioEdit 7.0.4.1. The optimized alignment file was analyzed by maximum parsimony (MP) in PAUP 4.0b10 (Swofford 2003). The analytical parameter preferences are described in Wu et al. (2007). Bootstrap analysis (Hillis & Bull 1993) was performed with 1,000 replicates with random addition sequences to obtain estimates of the reliability of the nodes.

Table 1. Taxa used in this study, along with their strain/specimen numbers, origins and GenBank accession numbers.

Species[A]	Strain/Specimen no.	Origin	GenBank accession no.
Calocera bambusicola	Wu 9910 12	Taipei, Taiwan	FJ195751
C. cornea	Wu 0606 1	Taichung, Taiwan	FJ195752
C. sinensis	Wu 0703 6	Nantou, Taiwan	FJ195754
C. sinensis	Wu 0505 3	Nantou, Taiwan	Wu 0505 3
C. sinensis	JCH 070726	Chiayi, Taiwan	FJ195755
C. viscosa	Wu 9905 30	Taichung, Taiwan	FJ195756
Dacrymyces sp.	FPL8953	USA	DQ205684
Guepiniopsis buccina	AFTOL ID 888	Washington, USA	DQ206986

Taxon names in bold indicate sequences from this study.

Taxonomy

Key to five species of Calocera with nodose-septate hyphae

1a. Basidiospores 1-septate 2
1b. Basidiospores 1-3-septate 4

2a. Basidiocarps tiny, shorter than 1.5 mm; contextual hyphae 1.5–3.3 µm diam.; basidiospores mostly narrower than 4.3 µm ..... *C. bambusicola*
2b. Basidiocarps 3–25 mm high; contextual hyphae 3–7.5 µm diam.; basidiospores wider than 4.3 µm 3

3a. Basidiocarps large, up to 25 mm high; clamped dikaryophyses lacking ..... *C. fusca* Lloyd

3b. Basidiocarps smaller, rarely exceeding 10 mm high; clamped dikaryophyses present ..... *C. sinensis*

4a. Basidiocarps cylindrical, spathulate, petaloid, or palmately lobed; basidiospores 11–15.5 µm long..... *C. guepinioides* Berk.

4b. Basidiocarps subulate or fusiform; basidiospores 13.5–17.5 µm long..... *C. macrospora* Brasf.

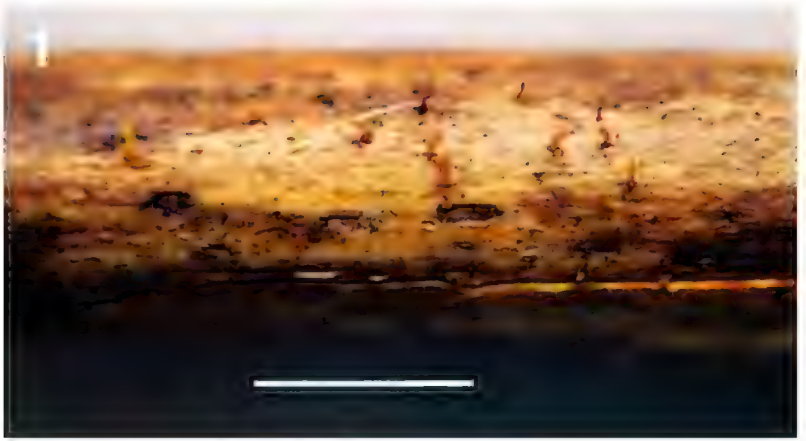

FIG. 1. Basidiocarps of *Calocera bambusicola* (*Wu 9910-10*). (scale bar = 1 cm).

Calocera bambusicola Sheng H. Wu, sp. nov. FIG. 1, 2 A–C

MYCOBANK MB 516557

Basidiocarpi gregarii, aurantiaci, simplices, clavati vel cylindrici, 0.1–0.3 mm diametro, ad 1.5 mm alti. Hyphae fibulatae. Cystidia desunt. Probasidia subclavata, 20–35 × 4–5 µm, deinde bifurcate. Basidiosporae ellipsoidea, 9–12 × 3.3–4.5 µm, 1-septatae, laeves, tenuitunicatae.

HOLOTYPE — Taiwan. Taipei City: Yangminshan National Park, Hsiaoyukeng, 25° 11' N 121°33'E, alt. 700 m, on culm of *Pseudosasa usawai*, leg. S.H. Wu et al., Oct. 23, 1999, Wu 9910-10 (TNM F9942).

ETYMOLOGY — referring to the habitat of this species.

Basidiocarps gregarious, yellow-orange when fresh, orange-brown when dry, gelatinous-cartilaginous when soaked, corneous when dry, simple, clavate or cylindrical, terete or slightly flattened, unbranched, apically blunt, 0.1–0.3 mm diam., up to 1.5 mm high. Hymenium amphigenous, surface smooth. Context with dense texture; hyphae nodose-septate, colorless, 1.5–3.3 µm diam., walls thin or up to 0.5 µm diam. Cystidia lacking. Probasidia subclavate, 20–35 × 4–5 µm, becoming bifurcate. Basidiospores ellipsoid, adaxially concave, slightly

curved, colorless, 9–12 × 3.3–4.5 µm, smooth, thin-walled, with one septum when mature, bearing a distinct apiculus, IKI-, germination not seen.

Ecology & Distribution — All specimens of *Calocera bambusicola* were collected from culms of *Pseudosasa usawai*, beside a sulfur spring; so far known only from northern Taiwan.

Additional specimens examined — Taiwan. Taipei City: Yangminshan National Park, Hsiaoyukeng, 25° 11' N 121°33'E, alt. 700 m, on culm of *Pseudosasa usawai*, leg. S.H. Wu et al., Oct. 23, 1999, *Wu 9910-12* (TNM F9943); Dec. 30, 1999, Wu *9912-19* (TNM F10187), Wu *9912-20* (TNM F10188), Wu *9912-24* (TNM F10189); Oct. 30, 2000, Wu *0010-205* (TNM F12263); Jun. 15, 2001, Wu *0106-18* (TNM F13446), Wu *0106-19* (TNM F13447), Wu *0106-24* (TNM F13449).

Remarks — *Calocera bambusicola* is distinct from other species of this genus in having very tiny basidiocarps and by growing on culms of bamboo. This new species resembles *C. sinensis* in having clamped hyphae and similar basidiospores, but differs in having much smaller basidiocarps, narrower basidiospores, and much narrower contextual hyphae.

Calocera sinensis McNabb, New Zealand J. Bot. 3: 36. 1965. Fig. 2 d–g

Basidiocarps gregarious, yellow to orange when fresh, orange-brown when dry, gelatinous-cartilaginous when soaked, corneous when dry, simple, cylindrical-clavate or spathulate, rarely branched, apically blunt or rarely pointed, 0.5–1.5 mm diam., up to 8 mm high. Hymenium amphigenous, surface smooth. Context with dense texture; hyphae nodose-septate, colorless, 3-7 µm diam., with 0.5–2 µm thick walls. Cystidia lacking. Hyphidia simple or branched. Probasidia subclavate, 25–40 × 3–4 µm, becoming bifurcate. Basidiospores ellipsoid, adaxially concave, slightly curved, colorless, 9–13 × 4.3–5.5 µm, smooth, thin-walled, with one septum when mature, bearing a distinct apiculus, IKI-, germination by conidia or by germ tubes.

Ecology & Distribution — This species grows on both gymnosperms and angiosperms in Taiwan, and causes a wood brown rot. Known from China (McNabb 1965, Peng et al. 1992) and Taiwan (this study).

Specimens examined — Taiwan. Taoyuan Hsien: Takuanshan, alt. 1,500 m, on log, leg. S.H. Wu & S.W. Chou, 20 Jul 1999, Wu *9907-7* (TNM F9939). MIAOLI HSIEN: Kuanwu-Hsuehchien, Peikenghsi Abandoned Trail, 24° 27' N 121°03' E, alt. 1800 m, on wood, leg. W.N. Chou, 9 Oct 1995, *CWN 01311* (TNM F4397). Taichung Hsien: Anmashan, 24° 16' N 120°00' E, alt. 2,250 m, on trunk of gymnosperm, leg. S.H. Wu & H.J. Chan, 8 Nov 1997, Wu *9711-30* (TNM F9382); on rotten trunk of angiosperm, leg. S.H. Wu et al., 10 May 1999, Wu *9905-25* (TNM F9937); Tashueishan Forest Road, alt. 1200 m, leg. S.Z. Chen, 31 May 1999, Chen *865* (TNM F9919). Nantou Hsien: Hsitou, 23° 40' N 120°47' E, alt. 1200 m, on stump of *Cryptomeria japonica*, leg. S.H. Wu, 19 Jun 1993, Wu *9306-11* (TNM F1054), Wu *9306-12* (TNM F1050); on stump of *Cryptomeria japonica*, leg. S.H. Wu, 27 Jul 1993, Wu *9307-78* (TNM F1243); on trunk of *Cryptomeria japonica*, leg. S.H. Wu, 6 Apr 1997, Wu *9704-19* (TNM F8604); on rotten wood of gymnosperm, leg. S.H.

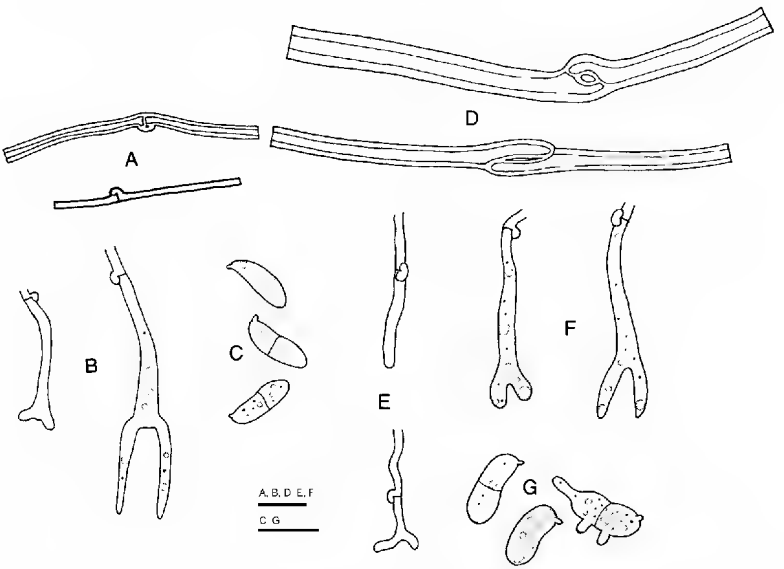

FIG. 2. *Calocera bambusicola* (*Wu 9910-10*: A C); *C. sinensis* (*Chen 865*: D G). Contextual hyphae (A, D); hyphidia (E); basidia (B, F); basidiospores (C, G). (scale bars 10 μm).

Wu, 3 May 2005, *Wu 0505-3* (TNM F18731); on branch of gymnosperm, leg. S.H. Wu & S.Z. Chen, 6 Jul 2007, Wu *0703-6* (TNM F20958); Sunlinhsi, 23° 38' N 120°47' E, alt. 1700 m, on fallen branch of *Cryptomeria japonica*, leg. S.H. Wu, 1 Jul 1992, Wu *9207-42* (TNM F0272); 19 Sep 1992, Wu *9209-88* (TNM F0361); Yushan National Park, Tatachia, 23° 29' N 120°54' E, alt. 2600 m, on decorticated branch of angiosperm, leg. S.H. Wu, 25 Nov 1993, Wu *9311-106* (TNM F1520). Ilan Hsien: Chilanshan, 24° 41' N 121°28' E, alt. 400 m, on wood of angiosperm, leg. S.H. Wu, 18 Nov 1992, Wu *9211-17* (TNM F0486).

REMARKS — *Calocera sinensis* has only been reported from China (McNabb 1965, Peng et al. 1992). In Taiwan, this species was collected from temperate to subtropical belts and is fairly common. *Calocera fusca* is allied to *C. sinensis*, but differs from the latter in having larger basidiocarps and lacks clamped dikaryophyses. *Calocera fusca* was reported from New Zealand, Australia, and Juan Fernández Islands (McNabb 1965), and China (Peng et al. 1992). However, further study should be performed to evaluate the importance of the features used for separating these two species.

Results and discussion on analysis of the ITS region

Amplification of the ITS region yielded PCR products approximately 530 bp long. The final alignment of the 8 sequences included 533 positions. Excluding the ambiguous sites at both ends, 452 sites were used for the MP analysis. Only

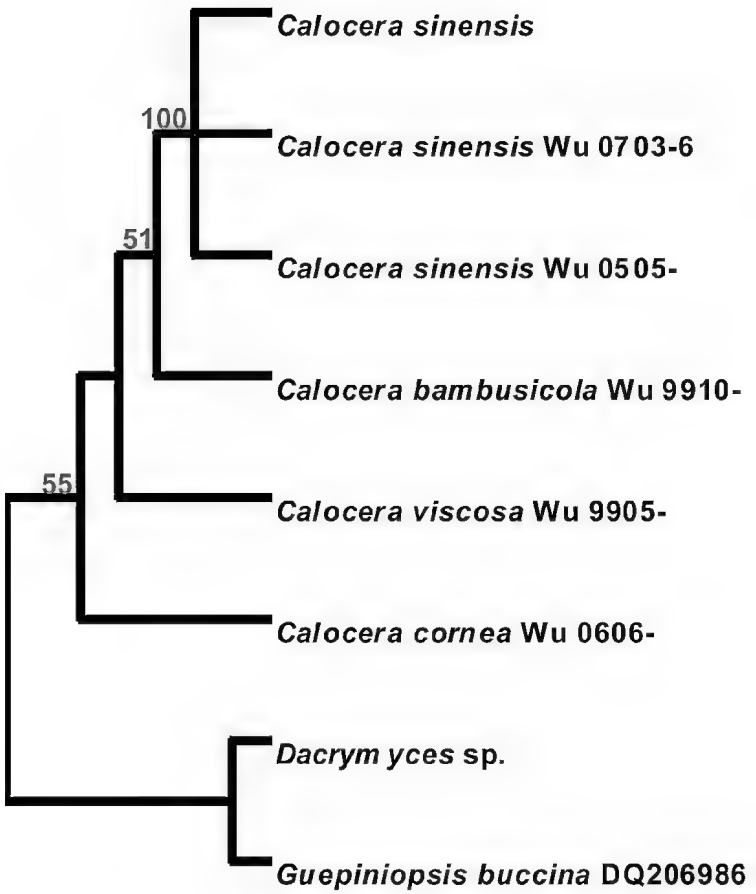

FIG. 3. The most parsimonious tree derived from ITS DNA sequence data. Bootstrap values are shown at nodes supported by no less than 50% from 1000 replicates. TL = 293, CI = 0.846, RI = 0.724.

one most parsimonious tree (293 steps, CI = 0.846, RI = 0.724) was retained in this analysis. Of the 452 included sites, 264 were constant, 96 were variable but parsimoniously uninformative, and 92 were parsimoniously informative. Bootstrap percentages were assigned above the branches. Three strains of *C. sinensis* were clustered together with 100% bootstrap support, and they were further clustered together with *C. bambusicola*, supported by a 51% bootstrap value (FIG 3).

The phylogenetic analysis indicates that the two species with clamp connections (*C. bambusicola, C. sinensis*) are more closely allied to each other than to two other species without clamp connections (*C. cornea, C. viscosa*). The three studied *C. sinensis* strains are identical in the alignment matrix of the 452 sites, after excluding the ambiguous sites at both ends. However, there were 32 transition, 49 transversion, and 29 deletions between *C. bambusicola* and *C. sinensis*, showing a 23.34% difference between the two species. Our ITS sequence analysis strongly supports *C. bambusicola* as independent from *C. sinensis*.

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The author is indebted to Dr. Nils Hallenberg and Dr. Peter Roberts for reviewing this paper, also to Ms. S.Z. Chen and Mr. W.N. Chou for contributing some collections for this study. This study was supported by the National Science Council of ROC (No. NSC 97-2621-B-178-001) and National Agriculture Council of ROC (No. 97AS-11.1.2-FB-e2).

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Stachybotrys subreniformis, new from soil in China

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Abstract — A new species of *Stachybotrys* from soil in China, *S. subreniformis*, is illustrated and described. It differs from similar species mainly by producing smaller conidia and shorter conidiophores. The type specimen (dried culture) and living culture are deposited in the Plant Pathology Herbarium of Guizhou University (HGUP).

Key words — soil fungi, taxonomy, dematiaceous hyphomycetes

Introduction

During an investigation of soil dematiaceous hyphomycetes in Guizhou Province, Southwestern China in 2009, one isolate possessing the typical characters of *Stachybotrys* Corda was obtained from forest soil and found to differ from described species of *Stachybotrys* in conidial and conidiophore morphology. The new taxon is illustrated and described below.

Materials & methods

Fifteen soil samples were collected from Duyun, Guizhou Province. The methods of sample collection, isolation and identification of fungi, and preparation of dried cultures were as previously reported by Jiang & Zhang (2007). Conidia and conidiophores were placed in a drop of 85% lactic acid and examined and photographed using light microscopy. Thirty mature conidia and phialides were measured by using a UB200i microscope (Chongqing UOP Photoelectric Technology Co., China) at 1000× magnification. The fungi are described from cultures grown at 25°C on corn meal agar (CMA).

Taxonomy

Stachybotrys subreniformis Q.R. Li & Y.L. Jiang, **sp. nov.** Fig. 1 a–b

MycoBank MB 519085

Coloniae effusae, densae, pilosae, griseae vel nigrae. Mycelium superficiale vel immersum. Hyphae hyalinae, ramosae, septatae, laeves, 5 7 µm crassae. Conidiophora macronemata, mononemata, erecta vel flexuosa, ramosa, septatae, hyalinae vel artobrunnea, 48 98 ×

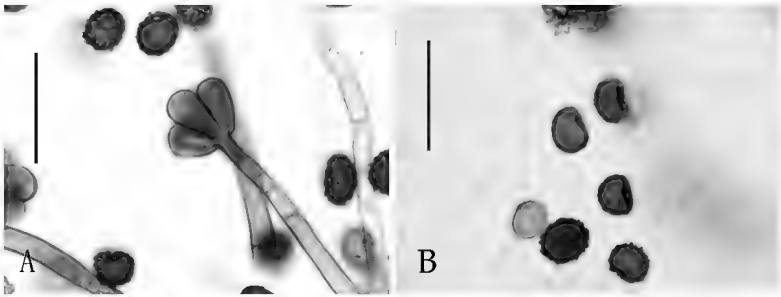

FIG. 1A–B. Conidia and conidiophores of *Stachybotrys subreniformis*. (Bars = 20 μm)

5.0–7.5 μm. Phialides brunnea, obovatae, laeves, 8.0–11.5 × 4.5–6.0 μm. Conidia sphaerica vel reniformia, aseptata, atrobrunnea, verruculosa, 6.0–9.5 × 4.5–7.5 μm.

HOLOTYPE: CHINA. GUIZHOU PROVINCE, Duyun, from forest soil, 15 December 2009, Q.R. Li, $HGUP_{09}1051$.

ETYMOLOGY: in reference to the slightly reniform shape of many of the conidia.

COLONIES on CMA effuse, dense, hairy, grey to black, colourless at first, then becoming dark with production of abundant anamorphic structures. MYCELIUM superficial and immersed: hyphae hyaline, branched, septate, smooth, 5–7 μm wide. CONIDIOPHORES macronematous, mononematous, erect or flexuous, branched, septate, hyaline to dark brown, 48–98 × 5.0–7.5 μm, tapering toward the apex which bears terminal phialides in a whorl of 2–7 around a central phialide. PHIALIDES brown, obovate, smooth, 8.0–11.5 × 4.5–6.0 (av. 9.3 ± 0.9 × 5.3 ± 0.4, n = 30) μm, with conspicuous collarettes. CONIDIA aggregated in slimy masses, spherical or slightly reniform, nonseptate, dark brown, verruculose, 6.0–9.5 × 4.5–7.5 (av. 7.6 ± 1.0 × 6.1 ± 0.8, n = 30) μm.

In the key to *Stachybotrys* species (Pinruan et al. 2004), seven species produced reniform conidia. They are *S. nephrodes* McKenzie (McKenzie 1991), *S. sinuatophora* Matsush. (Matsushima 1971), *S. reniverrucosa* Whitton et al. (Whitton et al. 2001), *S. nephrospora* Hansf. (Hansford 1943), *S. reniformis* Tubaki (Tubaki 1963) and *S. renisporoides* K.G. Karand. et al. (Karandikar et al. 1992) and *S. oenanthes* M.B. Ellis (Ellis 1971). However, only *S. oenanthes* produces ovoid to reniform conidia, which is very close to *S. subreniformis*. Conidia of *S. subreniformis* (6.0–9.5 × 4.5–7.5 μm) are smaller than those of *S. oenanthes* (9–12 × 4.5–8 μm). In addition, the conidiophores of *S. subreniformis* (48–98 μm) are shorter than those of *S. oenanthes* (up to 190 μm).

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New species *Koorchaloma* and *Ciliochorella* from xeric forests in Argentina

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Abstract— Two new species, *Koorchaloma scutiae* and *Ciliochorella buxifoliae*, isolated from leaf litter of *Scutia buxifolia* forest are described, illustrated, discussed, and compared with similar species. The specimens were collected in Magdalena, Buenos Aires province, Argentina. The type specimens are deposited in LPS. Keys are provided to all species of both genera.

Key words— anamorphic fungi, taxonomy

Introduction

Xeric forests dominated by *Scutia buxifolia* Reissek (*Rhamnaceae*) and *Celtis tala* Gillies ex Planch. (*Ulmaceae*) represent the most important woodland community ("La Pampa") in the eastern plains of Buenos Aires Province, Argentina (South America). They range from the banks of the Paraná River to the suburbs of Mar Chiquita town. The District of Magdalena has one of the most well preserved native forest areas, with an environmental heterogeneity that determines the variability in vegetation composition. Wooded ranges run parallel to the La Plata River, where the forest grows on highly calcareous substrates derived from Quaternary sea transgressions and regressions (Sánchez et al. 1976).

Several works have examined the fungal communities on decaying leaf litter of *Scutia buxifolia* and *Celtis tala* (Allegrucci et al. 2004, 2005, 2007; Crous et al. 2005). Yanna et al. (2001) noted that fungal species composition varies according to the different hosts and sites, which is particularly evident in the xeric forests of the Buenos Aires province (Allegrucci et al. 2009). This paper

describes two new species, *Koorchaloma scutiae* and *Ciliochorella buxifoliae*, recently recovered from *S. buxifolia* leaf litter during biodiversity surveys of this region.

Materials & methods

Study site— The study area lies in the Magdalena District, 20 km southeast of Magdalena town (35°11′S, 57°17′W) in the province of Buenos Aires, Argentina in a forested area dominated by *Scutia buxifolia* ("coronillo") and *Celtis tala* ("tala") and covering marine shell deposits that form parallel ridges along the coastline. See Arturi et al. (1997) for details on climate and forest ecology.

Sample Collection— Samples of leaf litter were collected from the forest, placed in zip-lock bags for transport to the laboratory, and stored in a refrigerator at 4°C until processed. Plant material was incubated at 25°C in Petri dish moist chambers (Elíades et al. 2007). Samples were examined over four weeks. Fungal species were examined with a Wild M8 stereo microscope and a Dialux 20 Leitz microscope.

Scanning Electron Microscopy— For the determination of fungal species in their natural substrate (leaf litter), material remained in formaldehyde at 1% for 4 hours and then postfixed with 2% formaldehyde for 12 hours. The fixative was removed by means of three consecutive washes with distilled water for subsequent dehydration. The fixing of the cultured material was modified with respect to time: a fixation was carried out in 1% formaldehyde for 3 hours and post-fixation in 2% formaldehyde for 4 hours. All the samples were then dehydrated in graded series of 30%, 50%, 70% and 100% ethanol (45 min in each concentration), the ethanol was replaced by liquid CO_2 and the samples dried by Critical Point (Blazers equipment, CP-30). All the samples were mounted onto double-sided carbon tape and gold plated in a Joel Fine Ion Sputter, JCF 100. Observations and photomicrographs were obtained with a Scanning Electron Microscope, JMS-T100, and with a JEOL Model 6340 LV Scanning Electron Microscope belonging to the Service of Electron Microscopy of the Museum of Natural Sciences of La Plata, Buenos Aires.

Taxonomy

Koorchaloma scutiae Alleg., Elíades & Aramb., **sp. nov.** Plate 1

MycoBank MB518290

Foliicola conidiomata stromatica, dispersa, alba vel flavida gelatinosa usque 40 60 µm diam. Stroma basale parva ex cellulis isodiametricibus, atrobrunneis vel brunneis compositae. Setae penitus conidiomatis intersparsae, sterile, solitariae, non-ramosae, erectae, rectae, laeves, crassitunicatae, septate, brunneae, ad epicem pallidorae 170 400 µm longae, 10 15 µm latae ad basem, 3 5 µm latem ad apicem. Conidiophora macronematosa, erecta, ramosa, tenuitunicata, laevia, hyalina. Cellulae conidiogenae discretae, monoblasticae, terminales, subcylindricae, cum collario, laeves hyalinae 10 15 × 2 2.7 µm. Conidia 18 20 × 1.8 2 µm fusiformia ad apice rotundata ad basim truncata, laevia hyalina utrique appendices mucosas ferentia, in masso gregaria.

Typus: *Argentina, Buenos Aires, Magdalena, in foliis mortuis Scutia buxifolia, Cazau 48245 (LPS,* ***holotypus****), May 2004*

Etymology: referring to substrate genus, *Scutia*.

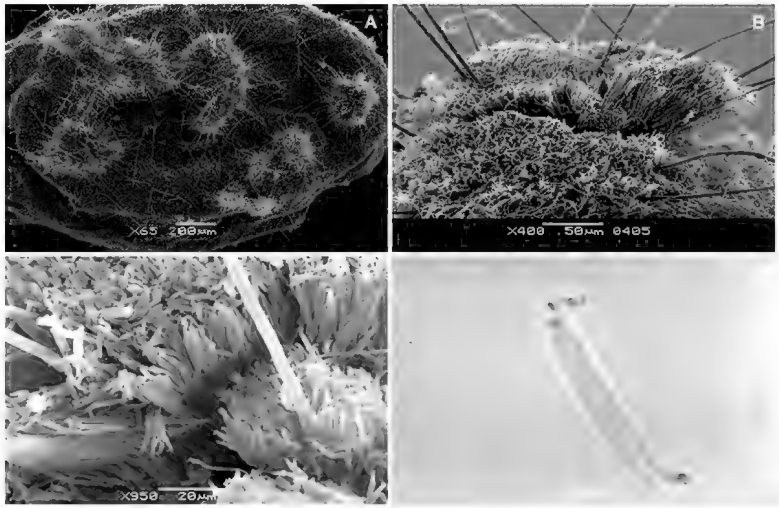

PLATE 1. *Koorchaloma scutiae.* A. Conidiomata on natural substrate. B. Detail of conidiomata. C. Conidiophores, conidiogenous cells, conidia and setae. D. Conidium.

Folicolous conidiomata stromatic, scattered, pulvinate, gelatinous, white to creamy, rounded or irregular in outline, setose, 40–60 µm diam. Basal stroma thin of textura angularis with 4–5 layers of thick walled cells, dark brown. No margin was observed. Conidiomatal setae marginal and frequently interspersed, through the basal stroma straight, smooth walls, subcylindrical, unbranched, multiseptate, thick walled dark brown at the base, pale brown in the apex, 170–400 µm at the terminal cell which has telescopic growing. Conidiophores arising from the upper layer of cells of the basal stroma, irregularly branched, septate, hyaline, invested in mucus.

Conidiogenous cells discrete, monoblastic, subcylindrical with flaring collarettes, colourless, smooth 10–15 × 2–27 µm. Conidia 18–20 × 1.8–2 µm fusiform to slightly sigmoid with rounded base and truncated apex, mucoid appendage bearing in both extremes, funnel shape, mean conidium length/width 10:1.

COMMENTS: *Koorchaloma* Subram. was proposed by Subramanian (1953) for the type species, *K. madreeya* Subram., collected on dead, rotting culms of *Oryza sativa* in India. The genus is characterized by sporodochial to acervular setose conidiomata (with setae either marginal or interposed through the basal stroma), holoblastic conidiogenesis, and unicellular, colourless, and fusiform conidia that bear mucoid appendages either only apically or at both ends (Nag Raj 1984, 1993). Nag Raj (1993) recognized five species: *K. madreeya*, *K. bambusae* Nag Raj, *K. jamaicensis* Nag Raj, *K. occidentalis* Nag Raj (Nag Raj,

1984), and *K. okamurae* I. Hino & Katum. (Hino 1961). Since this publication, five more species have been described — *K. novojournalis* Yanna et al. (Yanna et al. 1998), *K. galateae* Kohlm. & Volkm.-Kohlm. (Kohlmeyer & Volkmann-Kohlmeyer 2001), *K. spartinicola* V.V. Sarma et al. (Sarma et al. 2001), *K. dimorpha* Matsush. (Matsushima 2003), and *K. europaea* Treigienė (Treigienė 2006).

The new species, *K. scutiae,* which grows throughout the year on *Scutia buxifolia* leaf litter, is differentiated from other *Koorchaloma* species by its longer and wider conidia (length/width ratio = 10:1). Its macronematous conidiophores resemble those of *K. novojournalis,* which can be distinguished by shorter conidia (mean length/width ratio = 3.5:1). *Koorchaloma scutiae* is also resembles *K. jamaicensis*, described from grass blades in Jamaica, in setal structure and sporodochia, but the latter species produces distinctly broader conidia (mean conidium length/width ratio = 5.3:1).

Although two *Koorchaloma* species have *Kananascus* teleomorphs (Nag Raj 1984, 1993), no teleomorph is known for *Koorchaloma scutiae.*

Key to *Koorchaloma* species

1a. Marine habitat 2
1b. Non-marine habitat 3
2a. Mean conidium length/width ratio > 3.4:1 ..... *K. galateae*
2b. Mean conidium length/width ratio < 2.9:1 ..... *K. spartinicola*
3a. Species with *Acremonium* synanamorphosis ..... *K. dimorpha*
3b. Species without synanamorphosis 4
4a. Conidia bearing mucoid appendage usually at the apex only, conidioma not gelatinous 5
4b. Conidia bearing mucoid appendage usually at the apex only or at both ends, conidioma gelatinous 6
5a. Mean conidium length/width ratio < 2.8:1 ..... *K. okamurae*
5b. Mean conidium length/width ratio > 3.7:1 ..... *K. madreeya*
6a. Conidia bearing mucoid appendage usually at the apex only ..... *K. europaea*
6b. Conidia bearing mucoid appendage at both ends 7
7a. Conidia fusiform 8
7b. Conidia naviculate 9
8a. Conidiophores reduced to conidiogenous cells ..... *K. bambusae*
8b. Conidiophores macronematous 10
9a. Excipulum well developed; mean conidium length/width ratio > 5.3:1 *K. jamaicensis*
9b. Excipulum less developed; mean conidium length/width ratio < 3.9:1 *K. occidentalis*
10a. Mean conidium length/width ratio < 3.5:1 ..... *K. novojournalis*
10b. Mean conidium length/width ratio > 10:1 ..... *K. scutiae*

Ciliochorella buxifoliae Allegr., Elíades & Aramb., **sp. nov.** PLATE 2

MYCOBANK MB518291

Foliicola, conidiomata stromatica, dissita vel gregaria, origene subcuticularia, in sectione lenticula 300–500 µm lat, 140–150 µm alt., unilocularia, glabra, brunnea, paries 20–35 µm cr., e textura angulari compositus. Conidiophora e cellulis strati intimi telae basalis conidiomatum enascentia, simplicia et basi 1–2 septata vel ad cellulas conidiogenas redacta, in muco involuta. Cellulae conidiogenae holoblasticae, ageniformes, hyalinae, laeves, 3–3.5 × 4–5 µm. Conidia subfusiformia, triseptata, 19–21 × 2.5–2.7 µm, appendices ferentia; cellula basalis obconica, hyalina, laevis 2–3 µm long; cellulae medianae duae, cylindraceae, subhyalinae, aliquantum inaequalis 8 µm long, cellula apicalis hyalina, laevis, cassa, in appendicem apicalem singularem attenuata; appendix basalis singularis, excentrica simplex attenuata 3–5 µm long; ratione conidii long./lat. 7:1.

TYPUS: *Argentina, Buenos Aires, Magdalena, in foliis mortuis Scutia buxifolia, Allegrucci & Cazau 48244 (LPS* ***holotypus****) February 2005.*

ETYMOLOGY: referring to its leaf litter substrate species, *Scutia buxifolia.*

Folicolous conidiomata lenticular, stromatic, picnidioid scattered, subcuticular, conical in sectional view, 300–500 µm wide, 140–150 µm deep, unilocular, glabrous, pale brown, wall 20–35 µm thick, textura angularis, cells thin walled, pale brown to almost colourless with area of dehiscence ultimately breaking wide open. Conidiophores lining the cavity of the conidioma, unbranched, 1–2 septate at the base or reduced to conidiogenous cells, invested in mucus. Conidiogenous cells holoblastic, lageniform, hyaline, smooth 3–3.5 × 4–5 µm. Conidia subfusiform, slightly curved 3-septate 19–21 × 2.5–2.7 µm bearing appendages; basal cell obconical with a truncate base, hyaline, smooth, 2–3 µm long, devoid of contents; 2 median cells cylindrical, almost colourless, equal, 8 µm long, apical cell hyaline, smooth devoid of contents and modified into a branched appendage divided with 27–30 µm between both extremes; basal appendage single, eccentric, unbranched, attenuated, 3–5 µm long, mean conidium length/width ratio 7:1.

COMMENTS: The genus *Ciliochorella* Syd. was described in 1935 for the type species, *Ciliochorella mangiferae* (Sydow & Mitter 1935; see Sutton 1964 for a brief history of the genus). *Ciliochorella* is characterized by having cylindrical, straight or slightly curved conidia with euseptate pale brown middle cells, colourless end cells that bear a single, eccentric appendage. Nag Raj (1993) recognized three species: *C. splendida* Nag Raj & R.F. Castañeda (Nag Raj 1993), *C. castaneae* Munjal (Munjal, 1966), and *C. mangiferae* Syd. (Sydow & Mitter 1935). Although three other *Ciliochorella* species have been described, Nag Raj (1993) synonymized *C. eucalypti* T.S. Viswan. and *C. indica* Kalani with *C. mangiferae* and *C. bambusarum* Shanor with *Placonema bambusacearum* (Sacc. et al.) Petr.

The new species, *C. buxifoliae,* grows profusely throughout the year on *Scutia buxifolia* leaf litter. We placed our species into *Ciliochorella* because the

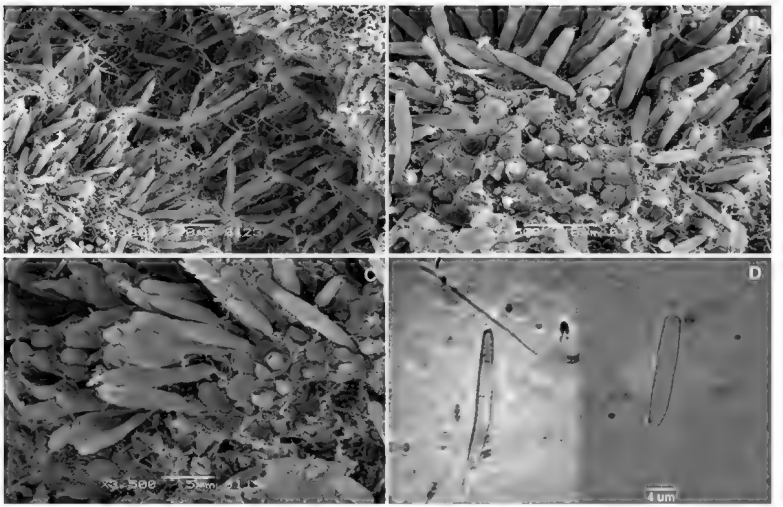

PLATE 2. *Ciliochorella buxifoliae*. A C. Conidiogenous cells and conidia. D. Conidium.

stromatic pycnidioid subcuticular adpressed to lenticular conidioma is never immersed in the host tissue; it differs from other *Cliochorella* species in size, septation, ramification, and form of the conidial appendage.

Ciliochorella buxifoliae differs from the three other species described above mainly by the longer and wider conidia. It most closely resembles *C. mangiferae,* which produces longer conidia (32–43 × 2.5–3.5 µm) with a mean conidium length/width ratio = 12.3:1.

Key to Ciliochorella species

1a. Conidiomata not lenticular ..... *C. splendida*
1b. Conidiomata lenticular 2
2a. Conidiophores 0–2 septate, branched occasionally reduced to conidiogenous cells ..... *C. castaneae*
2b. Conidiophores invariably reduced to conidiogenous cells 3
3a. Mean conidium length/width ratio 12.3:1 ..... *C. mangiferae*
3b. Mean conidium length/width ratio 7:1 ..... *C. buxifoliae*

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A new taxonomic classification for species in *Gomphus* sensu lato

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ABSTRACT Taxonomy of the *Gomphales* has been revisited by combining morphology and molecular data (DNA sequences) to provide a natural classification for the species of *Gomphus* sensu lato. Results indicate *Gomphus* s.l. to be non-monophyletic, leading to new combinations and the placement of its species into four genera: *Gomphus* sensu stricto (3 species), *Gloeocantharellus* (11 species), *Phaeoclavulina* (41 species), and *Turbinellus* (5 species).

KEY WORDS Fries, nomenclature, Persoon, systematics

Introduction

Gomphus sensu lato (*Gomphaceae*, *Gomphales*, *Basidiomycota*) is characterized by fleshy basidiomata that can have funnel- or fan-shaped pilei with wrinkled, decurrent hymenia. The genus, which was described by Persoon (1797a), has undergone several taxonomic and nomenclatural modifications over the past 200 years.

The taxonomy of *Gomphus* s.l. (*Gomphales*) has proven difficult because of the few reliable morphological characters available for classification. Consequently, species of *Gomphus* s.l. have been classified under *Cantharellus, Chloroneuron, Chlorophyllum, Craterellus, Gloeocantharellus, Nevrophyllum*, and *Turbinellus*.

A few species are mycorrhizal (Agerer et al. 1998, Bulakh 1978, Guzmán & Villarreal 1985, Khokhryakov 1956, Kropp & Trappe 1982, Masui 1926, 1927, Pantidou 1980, Trappe 1960, Valdés-Ramirez 1972). Some are considered rare

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and endangered, especially in the Pacific Northwestern USA (USDA/USDI 1994) and certain regions of Europe (Bulakh 1978, Bulakh & Govorova 2000, Dahlberg & Croneborg 2003, Kluzák 1994, Urbonas et al. 1990). *Gomphus bonarii, G. clavatus, G. floccosus*, and *G. kauffmanii* are listed as threatened in the 1994 Northwest Forest Plan (USDA/USDI 1994) because of their apparent close association with old-growth forests. *Gomphus clavatus* is considered rare (red-listed) in 17 countries of Europe (Dahlberg & Croneborg 2003).

Nomenclatural history of *Gomphus* sensu lato

The concept of *Gomphus* s.l. was established when Persoon (1796) first mentioned the name *Merulius*. Subsequently Persoon (1797b) proposed four new combinations, including *M. violaceus* (= *M. clavatus*). Persoon (1797a) also introduced the name *Gomphus* at the generic level, but did not assign species to his newly created genus; still the name was validly published. Later Persoon (1801) transferred *Gomphus* to *Merulius* section *Gomphus* with two documented taxa [*M. clavatus* var. *violaceus* and *M. clavatus* var. *spadiceus* (= *Clavaria truncata* Schmidel)] and afterward restored the genus rank to *Gomphus* (Persoon 1825) with five species, including *G. truncatus* (= *M. clavatus*). Gray (1821) accepted Persoon's genus name, which he assigned to one species, *Gomphus clavatus*. The genus has frequently been cited in the past as "*Gomphus* (Pers.) Gray" or "*Gomphus* Pers. ex Gray"; but once the starting date for fungal nomenclature was emended to 1753 (Voss et al. 1983), the correct citation has been *Gomphus* Pers.

Fries (1821) accepted *Gomphus* only at the infrageneric level, as *Cantharellus* 'tribus' *Gomphus* (not to be confused with the unrelated *Agaricus* subgen. *Gomphus* Fr.) and subsequently (Fries 1825) introduced the genus *Gomphora* as a nomenclatural synonym of *Gomphus* Pers.

Other authors involved with shaping the history of *Gomphus* s.l. included Schweinitz (1832), Berkeley (1839), Berkeley & Curtis (1859), Peck (1887), and Smith & Morse (1947), all of whom assigned gomphoid species to *Cantharellus*. Fries (1838) moved *Merulius clavatus* to *Craterellus*. Doassans & Patouillard (1886) described *Nevrophyllum* to accommodate *Gomphus* species, and Earle (1909) proposed *Turbinellus* to accommodate some species formerly assigned to *Cantharellus*.

Singer (1945) described *Gloeocantharellus* (based on *Cantharellus purpurascens*) with the key diagnostic character of gloeocystidia to separate it from *Cantharellus*. More importantly, here he also recognized *Gomphus* at the generic level, recombining *Cantharellus bonarii* as *G. bonarii*.

Corner (1950, 1966, 1970), and Petersen (1967, 1968, 1969, 1971, 1972), who clarified the natural classification of this group, accepted the classifications proposed by Donk (1933, 1941, 1949a,b), who also proposed the family

Gomphaceae (Donk 1961). The *Gomphaceae* was considered a member of the *Aphyllophorales* until Jülich (1981) proposed the order *Gomphales*.

The molecular overview of gomphoid-phalloid phylogeny by Hosaka et al. (2006) incorporated the data from Giachini (2004) for *Gomphus* s.l., including its four generic segregates. We here provide the conclusions from Giachini's research, a key to the segregate genera, and a list of accepted names, including new combinations, and synonyms.

Systematics, taxonomy, and evolutionary biology

Most difficulties in identifying taxa of *Gomphus* s.l. arise because of the few reliable morphological characters available for classification. However, distinctive clusters of species can be consistently separated by morphology. Most are united by a few consistent features, such as fan-shaped, lilaceous vs. funnel-shaped, orange-brown pilei, scaly vs. inconspicuously fibrous pileus surface, presence vs. absence of clamp connections, echinulate vs. verrucose spores, tropical vs. temperate habitat, etc.

Given the difficulty in identifying species of *Gomphus* s.l. by morphological characters, combined morphological and molecular data (DNA sequences) were used to verify the monophyly of *Gomphus* and to identify morphological features to reliably separate taxa within *Gomphus*.

Materials & methods

Collections examined

In all, 434 collections of *Gomphales* species were analyzed for distinct macro- and micro-morphological features, of which 189 were selected for molecular characterization (for details see Giachini 2004, Giachini et al. 2010).

Molecular characterization

Twenty-nine sequences of *Gomphus* s.l. representing 22 taxa were sampled for molecular characterization and phylogenetic analysis; DNA extraction, purification, sequencing, and analyses are according to Giachini (2004) and Giachini et al. (2010).

Results

Reexamination of generic types by morphological and molecular approaches revealed the inadequacy of some characters used by early workers to differentiate species within *Gomphus* s.l. Additionally, genetic data strongly suggest that *Gomphus* s.l. is not monophyletic (Giachini 2004, Giachini et al. 2010). Rather, it originated several times within the order *Gomphales* (Giachini et al. 2010: figures 1, 2). Because basidioma morphology and overall microscopic characteristics differentiate among species in many clades within *Gomphus* s.l., segregation of species into the genera *Gomphus* s.s., *Gloeocantharellus*, *Phaeoclavulina* (some species of *Gomphus* s.l. and all species of *Ramaria* subgenus *Echinoramaria*),

and *Turbinellus* is proposed. Detailed description of all species listed here will be published elsewhere.

Key to genera

1a. Basidiomata unipileate or merismatoid (composed of several pilei); pileus mostly funnel-shaped, surface almost glabrous to fibrillose, appressed-floccose, squamose scaly or with heavy recurved scales; clamp connections sometimes present but mostly absent; spores verrucose **2**

1b. Basidiomata ramarioid, unipileate or merismatoid; pileus, when present, fan- to funnel-shaped, surface glabrous; clamp connections always present; spores echinulate, verrucose, subreticulate or reticulate **3**

2a. Pileus minutely to strongly scaly, orange-yellow to orange-scarlet, blood red, pale orange, orange, or brown, often changing color on drying; scales generally darker than pileus surface; hymenium wrinkled ..... ***Turbinellus***

2b. Pileus glabrous to tomentose, off-white, pale yellow, pale yellow brown, yellow-pink, pale to livid orange, orange-pink, pink, pale brown, dark yellow-orange, dark rusty brown, or pale red; hymenium decurrent, supported by either true lamellae, bifurcating broad hymenial folds, or wrinkled folds ..... ***Gloeocantharellus***

3a. Basidiomata unipileate or merismatoid, terrestrial; pileus fan-shaped to slightly funnel-shaped, bright violet, pale olivaceous or brown; hymenium wrinkled to almost poroid, violet, vinaceous brown to milky-coffee colored; spores verrucose ..... ***Gomphus***

3b. Basidiomata ramarioid, unipileate or merismatoid, sometimes lignicolous; pileus, when present, funnel-shaped or fan-shaped, pileus and branch surfaces white, brown-green, pale to sordid olivaceous, violet, brown-yellow or red-cinnamon, gray, brick red, or pale to dark orange-yellow; hymenium smooth in some species, sublamellate or irregularly wrinkled with decurrent folds in others, in general yellow to orange-red or brown, pale to sordid olivaceous, or violet; spores echinulate (mostly with acute spines), verrucose, subreticulate or reticulate ..... ***Phaeoclavulina***

Accepted names and new combinations

New synonymies are prefixed with an asterisk * (based on Giachini et al. 2010)

Gomphus Pers. Tent. Disp. Meth. Fung.: 74 (1797)
[non *Gomphus* (Fr.) Weinm. 1826].

≡ *Merulius* sect. *Gomphus* Pers., Syn. Meth. Fung.: 498 (1801).

≡ *Cantharellus* 'trib.' *Gomphus* (Pers.) Fr., Syst. Mycol. 1: 322 (1821).

≡ *Gomphora* Fr., Syst. Orb. Veg.: 88 (1825).

≡ *Nevrophyllum* Pat., in Doassans & Patouillard, Revue Mycol. 8: 26 (1886), nom. illegit. [non *Neurophyllum* Torr. & A. Gray 1840].

≡ *Cantharellus* sect. *Gomphus* A.H. Sm. & Morse, Mycologia 39: 499 (1947).

Type species: *Gomphus clavatus* (Pers.) Gray 1821.

Gomphus brunneus (Heinem.) Corner, Ann. Bot. Mem. 2: 116 (1966).
BASIONYM: *Neurophyllum brunneum* Heinem., Bull. Jard. Bot. Etat. Brux. 28: 435 (1958).
Gomphus clavatus var. *parvisporus* Corner, Ann. Bot. Mem. 2: 118 (1966).

Gomphus clavatus (Pers.) Gray, Nat. Arr. Brit. Pl. 1: 638 (1821).
BASIONYM: *Merulius clavatus* Pers., Observ. Mycol. 1: 21 (1796).
≡ *Cantharellus clavatus* (Pers.) Fr., Syst. Mycol. 1: 322 (1821).
≡ *Craterellus clavatus* (Pers.) Fr., Epicr. Syst. Mycol.: 533 (1838).
≡ *Thelephora clavata* (Pers.) P. Kumm., Führ. Pilzk.: 46 (1871).
≡ *Nevrophyllum clavatum* (Pers.) Pat., in Doassans & Patouillard, Revue Mycol. 8: 26 (1886).
≡ *Trombetta clavata* (Pers.) Kuntze, Revis. Gen. Pl. 2: 873 (1891).
Clavaria elveloides Wulfen, Miscell. Austriaca Bot. 2: 99 (1781).
Clavaria truncata Schmidel , Anal. Pl. 3: 237 (1796).
≡ *Gomphus truncatus* (Schmidel) Pers., Mycol. Eur. 2: 9 (1825).
Merulius clavatus var. *spadiceus* Pers., Syn. Meth. Fung.: 498 (1801).
Cantharellus brevipes Peck, Rep. N. Y. St. Mus. Nat. Hist. 33: 21 (1883).
≡ *Merulius brevipes* (Peck) Kuntze, Revis. Gen. Pl. 2: 862 (1891).
Craterellus nucleatus Schröd., Centralbl. Gesammte Forstwes. 34: 396 (1908).
* *Cantharellus purpuraceus* Iwade, Bull. Tokyo Univ. Forests. 33: 54 (1944).
≡ *Gomphus purpuraceus* (Iwade) K. Yokoy., in Imazeki, Colour. Illustr. Mushr. Japan 2: 98 (1989).

Gomphus crassipes (L.M. Dufour) Maire, in Maire & Werner, Mém. Soc. Sci. Nat. Maroc 45: 81 (1937).
BASIONYM: *Cantharellus crassipes* L.M. Dufour, Rev. Gen. Bot. 1: 358 (1889).
≡ *Merulius crassipes* (L.M. Dufour) Kuntze, Revis. Gen. Pl. 3(2): 494 (1898).
≡ *Nevrophyllum crassipes* (L.M. Dufour) Maire, Bull. Soc. Mycol. Fr. 30: 214 (1914).

Gloeocantharellus Singer, Lloydia 8: 140 (1945).
Linderomyces Singer, Farlowia 3: 157 (1947).
Type species: *Cantharellus purpurascens* Hesler 1944.

Gloeocantharellus corneri (Singer) Corner, Nova Hedwigia 18: 799 (1970).
BASIONYM: *Linderomyces corneri* Singer, Vellozia 1: 14 (1961).

Gloeocantharellus dingleyae (Segedin) Giachini, **comb. nov.**
MYCOBANK MB512928
BASIONYM: *Gomphus dingleyae* Segedin, New Zealand J. Bot. 22: 536 (1985), as "*dingleyi*".
NOTE: The masculine termination '*-i*' is an error to be corrected to '*-ae*', because the epithet is a feminine possessive noun honouring Joan Dingley [see McNeill et al. 2006: Art. 32.7, 60.11 + Rec. 60C.1(a)].

Gloeocantharellus echinosporus Corner, Nova Hedwigia 18: 801 (1970).

Gloeocantharellus lateritius (Petch) Corner, Nova Hedwigia 18: 803 (1970).
BASIONYM: *Paxillus lateritius* Petch, Ann. Roy. Bot. Gard. Peradeniya 6: 202 (1917).

≡ *Phyllobolites lateritius* (Petch) Singer, Ann. Mycol. 40: 59 (1942).
≡ *Linderomyces lateritius* (Petch) Singer, Farlowia 3: 157 (1947).
≡ *Gomphus lateritius* (Petch) R.H. Petersen, J. Elisha Mitchell Scient. Soc. 84: 380 (1968).

Gloeocantharellus mamorensis (Singer) Giachini, **comb. nov.**
MycoBank MB 512929
Basionym: *Gomphus mamorensis* Singer, in Singer et al., Beih. Nova Hedwigia 77: 17 (1983).

Gloeocantharellus novae-zelandiae (Segedin) Giachini, **comb. nov.**
MycoBank MB512930
Basionym: *Gomphus novae-zelandiae* Segedin, New Zealand J. Bot. 22: 533 (1985).

Gloeocantharellus okapaensis (Corner) Corner, Nova Hedwigia 18: 803 (1970).
Basionym: *Gomphus okapaensis* Corner, Ann. Bot. Mem. 2: 123 (1966).

Gloeocantharellus pallidus (Yasuda) Giachini, **comb. nov.**
MycoBank MB512931
Basionym: *Cantharellus pallidus* Yasuda, in Lloyd, Mycol. Notes 47: 661 (1917).
≡ *Gomphus pallidus* (Yasuda) Corner, Ann. Bot. Mem. 2: 123 (1966).

Gloeocantharellus persicinus T.H. Li, Chun Y. Deng & L.M. Wu, in Deng & Li, Mycotaxon 106: 450 (2009).

Gloeocantharellus purpurascens (Hesler) Singer, Lloydia 8: 140 (1945).
Basionym: *Cantharellus purpurascens* Hesler, in Smith & Hesler, Lloydia 6: 248 (1944).
≡ *Gomphus purpurascens* (Hesler) R.H. Petersen, J. Elisha Mitchell Scient. Soc. 84: 380 (1968).

Gloeocantharellus uitotanus Vasco-Pal. & Franco-Mol., Mycotaxon 91: 90 (2005).

Phaeoclavulina Brinkmann, Jahresber. Westfäl. Provinz.-Ver. Wiss. Kunst 25: 197 (1897).
≡ *Clavaria* sect. *Phaeoclavulina* (Brinkmann) Killerm., in Engler & Prantl, Nat. Pflanzenfam. 6: 155 (1928).
* *Lachnocladium* sect. *Dendrocladium* Pat., J. Bot. Morot 3: 33 (1889).
≡ *Dendrocladium* (Pat.) Lloyd, Mycol. Notes 60: 870 (1919).
≡ *Ramaria* sect. *Dendrocladium* (Pat.) R.H. Petersen, Bibl. Mycol. 79: 44 (1981).
* *Chloroneuron* Murrill, Mycologia 3: 25 (1911).
≡ *Chlorophyllum* Murrill, North Amer. Flora 9: 172 (1910), nom. illeg. [non *Chlorophyllum* Massee 1898].
≡ *Neurophyllum* subgen. *Chloroneuron* (Murrill) R. Heim, Revue Mycol. 19: 51 (1954).
* *Clavaria* subsect. *Terrestres* Killerm., in Engler & Prantl, Nat. Pflanzenfam. 6: 154 (1928).
* *Ramaria* subgen. *Echinoramaria* Corner, Beih. Nova Hedwigia 33: 238 (1970).
* *Ramaria* ser. *Grandisporae* Corner, Beih. Nova Hedwigia 33: 239 (1970).
≡ *Ramaria* sect. *Grandisporae* (Corner) Franchi & M. Marchetti, Fungi non Delineati 16: 15 (2001).
* *Ramaria* ser. *Flaccidae* Corner, Beih. Nova Hedwigia 33: 239 (1970).
≡ *Ramaria* sect. *Flaccidae* (Corner) R.H. Petersen, Bibl. Mycol. 79: 100 (1981).

* *Ramaria* ser. *Virescentes* Corner, Beih. Nova Hedwigia 33: 239 (1970).

* *Gomphus* subgen. *Phaeoclavulinoides* R.H. Petersen, Mycologia 68: 432 (1976).

Type species: *Phaeoclavulina macrospora* Brinkmann 1897.

Phaeoclavulina abietina (Pers.) Giachini, **comb. nov.**

MycoBank MB512962

Basionym: *Clavaria abietina* Pers., Neues Mag. Bot. 1: 117 (1794) [sanctioned name; non "*Clavaria abietina*" sensu Fr. 1821].

≡ *Merisma abietinum* (Pers.) Spreng., Syst. Veg. 4: 495 (1827).

≡ *Hydnum abietinum* (Pers.) Duby, Bot. Gall 2: 778 (1830).

≡ *Clavariella abietina* (Pers.) P. Karst., Revue Mycol. 3(9): 21 (1880).

≡ *Ramaria abietina* (Pers.) Quél., Fl. Mycol. France: 467 (1888).

Clavaria ochraceovirens Jungh., Linnaea 5: 407 (1830).

≡ *Ramaria ochraceovirens* (Jungh.) Donk var. *ochraceovirens*, Meded. Ned. Mycol. Ver. 22: 112 (1933).

Clavaria abietina f. *persoonii* Fr., Hymenomyc. Eur.: 671 (1874).

Clavaria abietina var. *virens* Gillet, Tab. Anal.: 183 (1884).

* *Clavaria abietina* subsp. *cyanescens* Romell, in Krok & Almquist, Sv. Fl. Skol., Krypt., 2nd ed., (1911) [not seen; fide Corner 1950].

≡ *Clavaria cyanescens* (Romell) S. Lundell, in Krok & Almquist, Sv. Fl. Skol., Krypt., 5th ed.: 286 (1932) [not seen; fide Corner 1950].

Clavaria virescens Gramberg, Pilz- Kräuterfreund 5: 57 (1921).

≡ *Ramaria virescens* (Gramberg) Hennig, in Michael, Führ. Pilzfr. 3: 320 (1927).

* *Ramaria ochrochlora* Furrer-Ziogas & Schild, in Schild, Fung. Rar. Icon. Color. 5: 17 (1971).

* *Ramaria ochraceovirens* var. *parvispora* K.S. Thind, Khurana & S.C. Kaushal, Kavaka 11: 33 (1984).

Phaeoclavulina africana (R.H. Petersen) Giachini, **comb. nov.**

MycoBank MB512932

Basionym: *Gomphus africanus* R.H. Petersen, Mycologia 68: 429 (1976).

Phaeoclavulina angustata (Lév.) Giachini, **comb. nov.**

MycoBank MB512933

Basionym: *Merisma angustatum* Lév., Ann. Sci. Nat., Bot. sér. 3, 5: 158 (1846).

≡ *Thelephora angustata* (Lév.) Fr., Nova Acta Regiae Soc. Sci. Ups., Ser. 3, 1: 108 (1851).

≡ *Ramaria angustata* (Lév.) Corner, Beih. Nova Hedwigia 33: 242 (1970).

Phaeoclavulina apiahyna (Speg.) Giachini, **comb. nov.**

MycoBank MB512934

Basionym: *Clavaria apiahyna* Speg., Bol. Acad. Nac. Cienc. (Córdoba) 23: 435 (1919).

≡ *Ramaria apiahyana* (Speg.) Corner, Ann. Bot. Mem. 1: 555 (1950).

Phaeoclavulina argentea (R.H. Petersen) Giachini, **comb. nov.**

MycoBank MB512935

Basionym: *Ramaria argentea* R.H. Petersen, Bibl. Mycol. 79: 111 (1981).

* *Ramaria incognita* R.H. Petersen, Bibl. Mycol. 79: 145 (1981).

* *Ramaria incongrua* R.H. Petersen, Bibl. Mycol. 79: 147 (1981).

Phaeoclavulina articulotela (R.H. Petersen) Giachini, **comb. nov.**

MycoBank MB512936

Basionym: *Ramaria articulotela* R.H. Petersen, Bibl. Mycol. 79: 115 (1981).

Phaeoclavulina camellia (Corner) Giachini, **comb. nov.**

MycoBank MB512937

Basionym: *Ramaria camellia* Corner, Darwin. 11: 200 (1957).

Phaeoclavulina campestris (K. Yokoy. & Sagara) Giachini, comb. nov.

MycoBank MB512938

Basionym: *Ramaria zippelii* var. *campestris* K. Yokoy. & Sagara, Trans. Mycol. Soc. Japan 14: 302 (1973).

≡ *Ramaria campestris* (K. Yokoy. & Sagara) R.H. Petersen, Bibl. Mycol. 79: 57 (1981).

Phaeoclavulina campoi (Speg.) Giachini, **comb. nov.**

MycoBank MB512939

Basionym: *Clavaria campoi* Speg., Bol. Acad. Nac. Cienc. (Córdoba) 25: 28 (1921).

≡ *Ramaria campoi* (Speg.) R.H. Petersen, Bibl. Mycol. 79: 119 (1981).

Phaeoclavulina capucina (Pat.) Giachini, **comb. nov.**

MycoBank MB512940

Basionym: *Clavaria capucina* Pat., Bull. Soc. Mycol. Fr. 33: 50 (1917).

≡ *Ramaria capucina* (Pat.) Corner, Ann. Bot. Mem. 1: 565 (1950).

Phaeoclavulina cervicornis (A.L. Sm.) Giachini, **comb. nov.**

MycoBank MB512941

Basionym: *Clavaria cervicornis* A.L. Sm., J. Linn. Soc., Bot. 35: 10 (1901).

≡ *Ramaria cervicornis* (A.L. Sm.) Corner, Ann. Bot. Mem. 1: 565 (1950).

Phaeoclavulina clavarioides (Schild) Giachini, **comb. nov.**

MycoBank MB512942

Basionym: *Ramaria clavarioides* Schild, Z. Mykol. 64: 60 (1998).

Phaeoclavulina cokeri (R.H. Petersen) Giachini, **comb. nov.**

MycoBank MB512945

Basionym: *Ramaria cokeri* R.H. Petersen, Dist. Hist. Biota S. Appalachians 4: 291 (1976).

* *Clavaria echinospora* Berk. & Broome, J. Linn. Soc., Bot. 14: 75 (1873).

≡ *Scytinopogon echinosporus* (Berk. & Broome) Corner, Ann. Bot. Mem. 1: 655. (1950).

* *Clavaria fragillima* Sacc. & P. Syd., Syll. Fung. 16: 206 (1902).

≡ *Clavaria echinospora* Henn., Monsunia 1: 141 (1899), nom. illegit. [non *C. echinospora* Berk. & Broome 1873].

≡ *Clavariella fragillima* (Sacc. & P. Syd.) Overeem, Bull. Jard. Bot. Buitenzorg, ser. 3, 5: 275 (1923).

≡ *Ramaria fragillima* (Sacc. & Syd.) Corner, Ann. Bot. Mem. 1: 588 (1950).

Phaeoclavulina curta (Fr.) Giachini, **comb. nov.**

MycoBank MB512946

Basionym: *Clavaria curta* Fr., Öfvers. Förh. Kongl. Svenska Vetensk.-Akad. 18: 31 (1861).

≡ *Ramariopsis curta* (Fr.) Corner, Ann. Bot. Mem. 1: 639 (1950).

≡ *Ramaria curta* (Fr.) Schild, Z. Mykol. 60(1): 125 (1994).

Clavaria myceliosa Peck, Bull. Torrey Bot. Club 31(4): 182 (1904).
≡ *Ramaria myceliosa* (Peck) Corner var. *myceliosa*, Ann. Bot. Mem. 1: 607 (1950).
* *Clavaria decurrens* var. *australis* Coker, Clav. U.S.: 177 (1923).
≡ *Ramaria pusilla* var. *australis* (Coker) Corner, Ann. Bot. Mem. 1: 618 (1950).
≡ *Ramaria decurrens* var. *australis* (Coker) R.H. Petersen, Bibl. Mycol. 79: 127 (1981).
Ramaria pusilla Corner var. *pusilla*, Ann. Bot. Mem. 1: 617 (1950).
≡ *Clavaria pusilla* Peck, Ann. Rep. Reg. Univ. St. N.Y. 25: 83 (1873), nom. illegit. [non *C. pusilla* Pers. 1797].
≡ *Ramaria myceliosa* var. *microspora* R.H. Petersen, Bibl. Mycol. 79: 157 (1981).
* *Ramaria subdecurrens* var. *burnhamii* R.H. Petersen, Bibl. Mycol. 79: 171 (1981).

Phaeoclavulina cyanocephala (Berk. & M.A. Curtis) Giachini, **comb. nov.**

MycoBank MB512947
Basionym: *Clavaria cyanocephala* Berk. & M.A. Curtis, J. Linn. Soc., Bot. **10: 338 (1868).**
≡ *Ramaria cyanocephala* (Berk. & M.A. Curtis) Corner, Ann. Bot. Mem. 1: 568 (1950).
≡ *Ramaria grandis* f. *cyanocephala* (Berk. & M.A. Curtis) R.H. Petersen, Bibl. Mycol. 79: 71 (1981).
*= *Clavaria grandis* **Peck,** Bull. Torrey Bot. Club 29(2): 73 (1902).
≡ *Ramaria grandis* (Peck) Corner f. *grandis*, Ann. Bot. Mem. 1: 595 (1950).
≡ *Ramaria zippelii* f. *grandis* (Peck) R.H. Petersen, Taxonomy of Fungi, (Proc. int. Symp. Madras, 1973) Part 2: 569 (1984).
* *Clavaria spiculospora* G.F. Atk., **Ann. Mycol.** 7: 368 (1909) [cited as "*spiculisperma*" (lapsus calami) in Syll. Fung. 21: 426 (1912)].

Phaeoclavulina decolor (Berk. & M.A. Curtis) Giachini, **comb. nov.**

MycoBank MB512948
Basionym: *Clavaria decolor* Berk. & M.A. Curtis, Proc. Amer. Acad. Arts Sci. 4: 124 (1860).
≡ *Ramaria decolor* (Berk. & M.A. Curtis) R.H. Petersen, Persoonia 12(3): 231 (1984).
* *Ramaria zippelii* var. *cristatospora* Corner, Proc. Linn. Soc. London 178: 103 (1967).
≡ *Ramaria cristatospora* (Corner) R.H. Petersen, Bibl. Mycol. 79: 64 (1981).

Phaeoclavulina echinovirens (Corner, K.S. Thind & Dev) Giachini, **comb. nov.**

MycoBank MB512950
Basionym: *Ramaria echinovirens* Corner, K.S. Thind & Dev, Trans. Br. Mycol. Soc. 40: 473 (1957).

Phaeoclavulina eumorpha (P. Karst.) Giachini, **comb. nov.**

MycoBank MB512949
Basionym: *Clavariella spinulosa* subsp. *eumorpha* P. Karst., Bidr. Kännedom Finl. Natur Folk 37: 185 (1882).
≡ *Clavaria spinulosa* subsp. *eumorpha* (P. Karst.) Sacc., Syll. Fung. 6: 701 (1888).
≡ *Clavariella eumorpha* (P. Karst.) P. Karst., Bidr. Kännedom Finl. Natur Folk 48: 388 (1889).
≡ *Ramaria eumorpha* (P. Karst.) Corner, Ann. Bot. Mem. 1: 575 (1950).
* *Clavaria patagonica* Speg., Bol. Acad. Nac. Cienc. (Córdoba) 11: 21 (1887).
≡ *Clavulinopsis patagonica* (Speg.) Corner, Ann. Bot. Mem. 1: 383 (1950).
≡ *Ramaria patagonica* (Speg.) Corner, Darwin. 11: 203 (1957).

Clavaria invalii Cotton & Wakef., Trans. Br. Mycol. Soc. 6: 176 (1919).
≡ *Clavaria flaccida* subsp. *invalii* (Cotton & Wakef.) Konrad & Maubl., Icon. Select. Fung., fasc. 4: pl.488 (1928).
≡ *Ramaria invalii* (Cotton & Wakef.) Donk, Meded. Ned. Mycol. Ver. 22:113 (1933).
≡ *Clavaria flaccida* var. *invalii* (Cotton & Wakef.) J. Favre, Mater. Fl. Crypt. Suisse 10(3): 32 (1948).

* *Clavaria murrillii* Coker, Clav. U.S.: 190 (1923).
≡ *Ramaria murrillii* (Coker) Corner, Ann. Bot. Mem. 1: 607 (1950).

Phaeoclavulina flaccida (Fr.) Giachini, **comb. nov.**

MycoBank MB512951

Basionym: *Clavaria flaccida* Fr., Syst. Mycol. 1: 471 (1821).
≡ *Clavariella flaccida* (Fr.) P. Karst. var. *flaccida*, Revue Mycol. 3(9): 21 (1881).
≡ *Ramaria flaccida* (Fr.) Bourdot var. *flaccida*, Rev. Sci. Bourb. 11: 235 (1898).

* "*Clavaria abietina*" sensu Fr., Syst. Mycol. 1: 469 (1821), nom. inval. [non *Clavaria abietina* Pers. 1794].

* *Clavaria muscicola* Pers., Observ. Mycol. 2: 60 (1800).
≡ *Pistillaria muscicola* (Pers.) Fr., Syst. Mycol. 1: 498 (1821).
≡ *Typhula muscicola* (Pers.) Fr., Epicr. Syst. Mycol.: 585 (1838).
≡ *Eocronartium muscicola* (Pers.) Fitzp., Phytopathology 8: 197 (1918).

* *Clavaria crispula* Fr., Syst. Mycol. 1: 470 (1821).
≡ *Merisma crispulum* (Fr.) Spreng. Syst. Veg. 4: 496 (1827).
≡ *Clavariella crispula* (Fr.) P. Karst., Revue Mycol. 3(9): 21 (1881).
≡ *Ramaria crispula* (Fr.) Quél., Fl. Mycol. France: 464 (1888).
≡ *Ramaria flaccida* var. *crispula* (Fr.) Schild, Hoppea 61: 317 (2000).

* *Clavaria decurrens* Pers. Mycol. Eur. 1: 164 (1822).
≡ *Ramaria decurrens* (Pers.) R.H. Petersen var. *decurrens*, Bibl. Mycol. 79: 124 (1981).

* *Clavaria corrugata* P. Karst., Not. Sällsk. Fauna Fl. Fenn. Förh. 9: 371 (1868).
≡ *Clavariella corrugata* (P. Karst.) P. Karst., Revue Mycol. 3(9): 21 (1881).
≡ *Clavaria abietina* f. *corrugata* (P. Karst.) Killerm., Denkschr. Bayr. Bot. Ges. Regensburg 15: 113 (1922).
≡ *Ramaria corrugata* (P. Karst.) Schild, Schweiz. Z. Pilzk. 53: 130 (1975).

* *Clavariella flaccida* var. *dendroides* P. Karst., Not. Sällsk. Fauna Fl. Fenn. Förh. 9: 372 (1868).

* *Clavaria muscigena* P. Karst., Not. Sällsk. Fauna Fl. Fenn. Förh. 9: 373 (1868).
≡ *Clavaria uncialis* subsp. *muscigena* (P. Karst.) Sacc., Syll. Fung. 6: 730 (1888).
≡ *Eocronartium muscigena* (P. Karst.) Höhn., Sitzungsber. K. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 118: 1463 (1909).

* *Eocronartium typhuloides* G.F. Atk., J. Mycol. 8: 107 (1902).
≡ *Helicobasidium typhuloides* (G.F. Atk.) Pat., Bull. Soc. Mycol. Fr. 36: 176 (1920).

* *Clavaria subdecurrens* Coker var. *subdecurrens*, Clav. U.S.: 172 (1923).
≡ *Ramaria subdecurrens* (Coker) Corner var. *subdecurrens*, Ann. Bot. Mem. 1: 626 (1950).

* *Ramaria ligustri* Velen., Novit. Mycol.: 162 (1939).

* *Ramaria fuscobrunnea* Corner, in Balfour-Browne, Bull. Brit. Mus. Nat. Hist. 1: 200 (1955).

* *Ramaria flaccida* var. *longiramosa* Corner, Beih. Nova Hedwigia 33: 250 (1970).

* *Ramaria perfluopunicea* R.H. Petersen, New Zealand DSIR Bull. 236: 100 (1988).

Phaeoclavulina gigantea (Pat.) Giachini, **comb. nov.**

MycoBank MB512952

Basionym: *Lachnocladium giganteum* Pat., J. Bot. (Morot) 3: 34 (1889).

≡ *Ramaria gigantea* (Pat.) R.H. Petersen f. *gigantea*, Bibl. Mycol. 79: 66 (1981).

Lachnocladium olivaceum Henn., Hedw. 36: 196 (1897).

Lachnocladium echinosporum Bres., Ann. Mycol. 9: 551 (1911).

* *Ramaria zippelii* var. *gracilis* Corner, Ann. Bot. Mem. 1: 635, 700 (1950).

* *Ramaria gigantea* f. *tenuispora* R.H. Petersen, New Zealand DSIR Bull. 236: 97 (1988).

Phaeoclavulina glauco-aromatica (R.H. Petersen) Giachini, **comb. nov.**

MycoBank MB512953

Basionym: *Ramaria glauco-aromatica* R.H. Petersen, Bibl. Mycol. 79: 141 (1981).

Phaeoclavulina grandis (Corner) Giachini, **comb. nov.**

MycoBank MB512954

Basionym: *Gomphus grandis* Corner, Nova Hedwigia 18: 812 (1970).

Phaeoclavulina griseobrunnea (Singer) Giachini, **comb. nov.**

MycoBank MB512955

Basionym: *Ramaria griseobrunnea* Singer, in Singer et al., Beih. Nova Hedwigia 77: 19 (1983).

Phaeoclavulina guadelupensis (Pat.) Giachini, **comb. nov.**

MycoBank MB512956

Basionym: *Stereum guadelupense* Pat., Bull. Soc. Mycol. Fr. 15: 201 (1899).

≡ *Gomphus guadelupensis* (Pat.) D.A. Reid, Persoonia 2: 134 (1962).

* *Nevrophyllum ochraceum* Pat., in Duss, Énum. Champ. Guadeloupe: 38 (1903).

≡ *Cantharellus ochraceus* (Pat.) Sacc. & D. Sacc., Syll. Fung. 17: 36 (1905).

≡ *Gomphus ochraceus* (Pat.) Singer, Lloydia 8: 140 (1945).

Phaeoclavulina guyanensis (Pat.) Giachini, **comb. nov.**

MycoBank MB512957

Basionym: *Lachnocladium guyanense* Pat., J. Bot. (Morot) 3: 35 (1889).

≡ *Ramaria guyanensis* (Pat.) Corner, Ann. Bot. Mem. 1: 596 (1950).

≡ *Clavaria guyanensis* (Pat.) Rick, Iheringia 5: 132 (1959).

* *Lachnocladium leucoceras* Pat., J. Bot. (Morot) 3: 33 (1889).

≡ *Ramaria leucoceras* (Pat.) Corner, Ann. Bot. Mem. 1: 600 (1950).

Phaeoclavulina insignis (Pat.) Giachini, **comb. nov.**

MycoBank MB512958

Basionym: *Lachnocladium insigne* Pat., J. Bot. (Morot) 3: 34 (1889).

≡ *Ramaria insignis* (Pat.) R.H. Petersen, Bibl. Mycol. 79: 77 (1981), as "*insigne*".

Phaeoclavulina longicaulis (Peck) Giachini, **comb. nov.**

MycoBank MB512959

Basionym: *Clavaria longicaulis* Peck, Bull. Torrey Bot. Club 25: 371 (1898).

≡ *Ramaria longicaulis* (Peck) Corner, Ann. Bot. Mem. 1: 600 (1950).

Phaeoclavulina macrospora Brinkmann, Jber. Westf. Prov. Ver. Wiss. Kunst 25: 198 (1897).

≡ *Ramaria macrospora* (Brinkmann) Corner, Ann. Bot. Mem. 1: 603 (1950), nom. illegit. [non *R. macrospora* Velen. 1947].

Phaeoclavulina nigrescens Brinkmann, Jber. Westf. Prov. Ver. Wiss. Kunst 25: 198 (1897).

≡ *Ramaria nigrescens* (Brinkmann) Donk var. *nigrescens*, Meded. Ned. Mycol. Ver. 22: 104 (1933).

Clavaria broomei Cotton & Wakef., Trans. Br. Mycol. Soc. 6: 170 (1919).

≡ *Phaeoclavulina broomei* (Cotton & Wakef.) Overeem, Bull. Jard. Bot. Buitenzorg, ser. 3, 5: 277 (1923).

≡ *Ramaria broomei* (Cotton & Wakef.) R.H. Petersen, Bibl. Mycol. 79: 53 (1981).

* *Ramaria nigrescens* var. *americana* Corner, Ann. Bot. Mem. 1: 608, 700 (1950).

≡ *Ramaria americana* (Corner) R.H. Petersen, Bibl. Mycol. 79: 48 (1981).

Phaeoclavulina mutabilis (Schild & R.H. Petersen) Giachini, **comb. nov.**

MycoBank MB512960

Basionym: *Ramaria mutabilis* Schild & R.H. Petersen, in Petersen, Bibl. Mycol. 79: 149 (1981).

Phaeoclavulina ochracea (Bres.) Giachini, **comb. nov.**

MycoBank MB512961

Basionym: *Lachnocladium ochraceum* Bres., in Bresadola & Saccardo, **Bull. Soc. Roy. Bot. Belgique 38: 157 (1899).**

≡ *Ramaria ochracea* (Bres.) Corner var. *ochracea*, Ann. Bot. Mem. 1: 610 (1950).

* *Clavaria intricatissima* Speg., Anales Mus. Nac. Buenos Aires 19: 279 (1909).

≡ *Ramaria intricatissima* (Speg.) Corner, Ann. Bot. Mem. 1: 598 (1950).

* *Ramaria luteoflaccida* Corner, Ann. Bot. Mem. 1: 601, 699 (1950).

* *Ramaria flaccida* var. *chilensis* Lazo, Mycol. 64: 77 (1972).

* *Ramaria ochracea* var. *sicco-olivacea* R.H. Petersen, New Zealand DSIR Bull. 236: 98 (1988).

Phaeoclavulina pancaribbea (R.H. Petersen) Giachini, **comb. nov.**

MycoBank MB512963

Basionym: *Ramaria pancaribbea* R.H. Petersen f. *pancaribbea*, Bibl. Mycol. 79: 88 (1981).

* *Ramaria pancaribbea* f. *caerulea* R.H. Petersen, Bibl. Mycol. 79: 90 (1981).

Phaeoclavulina quercus-ilicis (Schild) Giachini, **comb. nov.**

MycoBank MB512964

Basionym: *Ramaria quercus-ilicis* Schild, Z. Mykol. 64: 53 (1998).

Phaeoclavulina retispora (Corner) Giachini, **comb. nov.**

MycoBank MB512965

Basionym: *Gomphus retisporus* Corner, Ann. Bot. Mem. 2: 124 (1966).

Phaeoclavulina roellinii (Schild) Giachini, **comb. nov.**

MycoBank MB512966

Basionym: *Ramaria roellinii* Schild, Schweiz. Z. Pilzk. 56: 97 (1978).

Phaeoclavulina sikkimia (S.S. Rattan & Khurana) Giachini, **comb. nov.**

MycoBank MB512967

Basionym: *Ramaria sikkimia* S.S. Rattan & Khurana, Bibl. Mycol. 66: 18 (1978).

Phaeoclavulina subclaviformis (Berk.) Giachini, **comb. nov.**

MycoBank MB512968

Basionym: *Thelephora subclaviformis* Berk., Hooker's J. Bot. Kew Gard. Misc. 8: 238 (1856), as "*subclavaeformis*".

Note: The '*-ae-*' connective is an error, to be replaced with an '*-i-*' (McNeill et al. 2006: Art. 60.8).

≡ *Gomphus subclaviformis* (Berk.) Corner, Nova Hedwigia 18: 815 (1970), as "*subclavaeformis*".

* *Gomphus cavipes* Corner, Nova Hedwigia 18: 808 (1970).

Phaeoclavulina tropicalis (R.H. Petersen) Giachini, **comb. nov.**

MycoBank MB512969

Basionym: *Ramaria tropicalis* R.H. Petersen, Bibl. Mycol. 79: 173 (1981).

Phaeoclavulina vinaceipes (Schild) Giachini, **comb. nov.**

MycoBank MB512970

Basionym: *Ramaria vinaceipes* Schild, Z. Mykol. 56(1): 131 (1990).

Phaeoclavulina viridis (Pat.) Giachini, **comb. nov.**

MycoBank MB512971

Basionym: *Nevrophyllum viride* Pat., J. Bot. (Morot) 2: 406 (1888).

≡ *Chlorophyllum viride* (Pat.) Murrill, North Amer. Flora 9(3): 172 (1910).

≡ *Chloroneuron viride* (Pat.) Murrill, Mycologia 3(1): 25 (1911).

≡ *Gomphus viridis* (Pat.) Singer, Lloydia 8: 140 (1945).

* *Cantharellus patouillardii* Sacc., Syll. Fung. 9: 65 (1891).

≡ *Merulius patouillardii* (Sacc.) Kuntze, Revis. Gen. Pl. 3(2): 494 (1898).

Phaeoclavulina zealandica (R.H. Petersen) Giachini, **comb. nov.**

MycoBank MB512972

Basionym: *Ramaria pancaribbea* var. *zealandica* R.H. Petersen, New Zealand DSIR Bull. 236: 99 (1988).

Phaeoclavulina zippelii (Lév.) Overeem, Bull. Jard. Bot. Buitenzorg, ser. 3, 5: 276 (1923).

Basionym: *Clavaria zippelii* Lév., Ann. Sci. Nat., Bot. sér. 3, 2: 215 (1844).

≡ *Ramaria zippelii* (Lév.) Corner f. *zippelii*, Ann. Bot. Mem. 1: 632 (1950).

* *Merisma guadelupense* Lév., Ann. Sci. Nat., Bot. sér. 3, 5: 157 (1846).

≡ *Pterula guadelupensis* (Lév.) Sacc., Syll. Fung. 6: 742 (1888).

≡ *Lachnocladium guadelupense* (Lév.) Pat., J. Bot. (Morot) 3: 33 (1889).

≡ *Microporus guadelupensis* (Lév.) Kuntze, Revis. Gen. Pl. 3(2): 496 (1898).

≡ *Dendrocladium guadelupense* (Lév.) Lloyd, Mycol. Writ. 5(Index): 13 (1919), as "*guadeloupe*".

≡ *Ramaria guadelupensis* (Lév.) Corner, Ann. Bot. Mem. 1: 596 (1950).

≡ *Aphelaria guadelupensis* (Lév.) Corner, Beih. Nova Hedwigia 33: 19 (1970).

* *Clavaria nigrescens* Fr., Nova Acta Regiae Soc. Sci. Ups., Ser. 3, 1: 116 (1851).

*= *Lachnocladium hookeri* **Berk.,** Hooker's J. Bot. Kew Gard. Misc. 4: 140 (1852).

Clavaria thwaitesii Berk. & Broome, J. Linn. Soc., Bot. 14: 75 **(1873).**

Clavaria aeruginosa Pat., Bull. Soc. Mycol. Fr. 14: 189 (1898).

≡ *Ramaria zippellii* var. *aeruginosa* (Pat.) R.H. Petersen, Bibl. Mycol. 79: 96. 1981.

Clavaria phaeocladia Pat., Bull. Soc. Mycol. Fr. 14: 189 (1898).
= *Lachnocladium albidum* Pat., Bull. Soc. Mycol. Fr. 14: 188 (1898).
* *Lachnocladium englerianum* Henn., Bot. Jahrb. Syst. 25: 499 (1898).
* *Lachnocladium neglectum* Massee, in Wakefield et al., Vierteljahrsschr. Naturf. Ges. Zürich 61: 630 (1916).

Turbinellus Earle, Bull. N. Y. Bot. Gard. 5: 407 (1909).

Cantharellus sect. *Excavatus* A.H. Sm. & Morse, Mycologia 39: 500 (1947).
≡ *Gomphus* sect. *Excavatus* (A.H. Sm. & Morse) Corner, Ann. Bot. Mem. 2: 113 (1966).
≡ *Gomphus* subgen. *Excavatus* (A.H. Sm. & Morse) R.H. Petersen, J. Elisha Mitchell Scient. Soc. 84: 381 (1968).

Type species: *Cantharellus floccosus* Schwein. 1832.

Turbinellus flabellatus (Berk.) Giachini, **comb. nov.**

MycoBank MB512973

Basionym: *Cantharellus flabellatus* Berk., J. Linn. Soc., Bot. 16: 50 (1877).
≡ *Merulius flabellatus* (Berk.) Kuntze, Revis. Gen. Pl. 3(2): 494 (1898).
≡ *Gomphus flabellatus* (Berk.) Corner, Ann. Bot. Mem. 2: 119 (1966).
* *Cantharellus glutinosus* Pat., Bull. Soc. Mycol. Fr. 25: 7 (1909).
≡ *Gomphus glutinosus* (Pat.) R.H. Petersen, Nova Hedwigia 21: 92 (1972).

Turbinellus floccosus (Schwein.) Earle, Bull. N.Y. Bot. Gard. 5: 407 (1909).

Basionym: *Cantharellus floccosus* Schwein. f. *floccosus*, Trans. Amer. Phil. Soc. 4: 153 (1832).
≡ *Merulius floccosus* (Schwein.) Kuntze, Revis. Gen. Pl. 2: 862 (1891).
≡ *Chanterel floccosus* (Schwein.) Murrill, N. Amer. Flora 9(3): 168 (1910).
≡ *Gomphus floccosus* (Schwein.) Singer f. *floccosus*, Lloydia 8: 140 (1945).
≡ *Nevrophyllum floccosum* (Schwein.) R. Heim, Revue Mycol. 19: 51 (1954).
Cantharellus canadensis Klotzsch, in Berkeley, Ann. Nat. Hist. 3: 380 (1839).
≡ *Craterellus canadensis* (Klotzsch) Sacc., Syll. Fung. 6: 519 (1888).
≡ *Trombetta canadensis* (Klotzsch) Kuntze, Revis. Gen. Pl. 2: 873 (1891).
≡ *Gomphus canadensis* (Klotzsch) Corner, Ann. Bot. Mem. 2: 116 (1966).
Cantharellus princeps Berk. & M.A. Curtis, Ann. Mag. Nat. Hist., ser. 3, 4: 293 (1859).
≡ *Merulius princeps* (Berk. & M.A. Curtis) Kuntze, Revis. Gen. Pl. 2: 862 (1891).
* *Cantharellus bonarii* Morse, Mycologia 22: 219 (1930).
≡ *Gomphus bonarii* (Morse) Singer f. *bonarii*, Lloydia 8: 140 (1945).
* *Cantharellus floccosus* f. *excavatus* A.H. Sm. & Morse, Mycologia 39: 525 (1947).
≡ *Gomphus floccosus* f. *excavatus* (A.H. Sm. & Morse) Corner, Ann. Bot. Mem. 2: 121 (1966).
* *Cantharellus floccosus* f. *rainieriensis* A.H. Sm. & Morse, Mycologia 39: 521 (1947).
≡ *Gomphus floccosus* f. *rainieriensis* (A.H. Sm. & Morse) Corner, Ann. Bot. Mem. 2: 121 (1966), as "*raineriensis*".
≡ *Gomphus floccosus* subsp. *rainieriensis* (A.H. Sm. & Morse) R.H. Petersen, Nova Hedwigia 21: 48 (1972).
* *Cantharellus floccosus* f. *wilsonii* A.H. Sm. & Morse, Mycologia 39: 523 (1947).
≡ *Gomphus floccosus* f. *wilsonii* (A.H. Sm. & Morse) Corner, Ann. Bot. Mem. 2: 121 (1966).

≡ *Gomphus bonarii* f. *wilsonii* (A.H. Sm. & Morse) R.H. Petersen, Nova Hedwigia 21: 37 (1972).

* *Cantharellus wilkinsiae* Morse, in Smith & Morse, Mycologia 39: 528 (1947), as "*wilkinsae*".

≡ *Gomphus wilkinsiae* (Morse) Corner, Ann. Bot. Mem. 2: 128 (1966), as "*wilkinsae*".

≡ *Gomphus bonarii* f. *wilkinsiae* (Morse) R.H. Petersen, Nova Hedwigia 21: 35 (1972), as "*wilkinsae*".

* *Gomphus floccosus* f. *cystidiophorus* R.H. Petersen, J. Elisha Mitchell Scient. Soc. 84: 376 (1968).

* *Gomphus megasporus* Corner, Nova Hedwigia 18: 813 (1970).

* *Gomphus bonarii* f. *novamexicanus* R.H. Petersen, Nova Hedwigia 21: 44 (1972).

* *Gomphus floccosus* f. *coccineobasalis* R.H. Petersen, Nova Hedwigia 21: 52 (1972), as "*coccineo-basalis*".

* *Gomphus floccosus* f. *olivaceus* R.H. Petersen, Nova Hedwigia 21: 54 (1972).

* *Gomphus szechwanensis* R.H. Petersen, Nova Hedwigia 21: 102 (1972).

* *Gomphus thiersii* R.H. Petersen, Evol. Higher Basidio.: 363 (1971).

Turbinellus fujisanensis (S. Imai) Giachini, **comb. nov.**

MycoBank MB512974

Basionym: *Cantharellus fujisanensis* S. Imai, Bot. Mag.Tokyo 55: 519 (1941).

≡ *Nevrophyllum fujisanense* (S. Imai) S. Ito, Mycol. Fl. Japan 2(4): 103 (1955), as "*fujisanensis*".

≡ *Gomphus fujisanensis* (S. Imai) Parmasto, Ident. URSS Clav.: 28 (1965).

Turbinellus kauffmanii (A.H. Sm.) Giachini, **comb. nov.**

MycoBank MB512975

Basionym: *Cantharellus kauffmanii* A.H. Sm., in Smith & Morse, Mycologia 39: 516 (1947).

≡ *Gomphus kauffmanii* (A.H. Sm.) Corner, Ann. Bot. Mem. 2: 122 (1966).

Turbinellus stereoides (Corner) Giachini, **comb. nov.**

MycoBank MB512976

Basionym: *Gomphus stereoides* Corner, Ann. Bot. Mem. 2: 124 (1966).

* *Gomphus brasiliensis* Corner, Nova Hedwigia 18: 807 (1970).

Discussion

Hibbett et al. (1997), Pine et al. (1999), Humpert et al. (2001), Giachini (2004), and now Giachini et al. (2010) have shown that evolutionarily the genus *Ramaria,* long recognized as having morphological features similar to *Gomphus* s.l., is closely related to species of that genus. Aside from the overall evolutionary relationships of *Gomphus* s.l. and *Ramaria*, Giachini (2004) revealed in particular a closer relationship between some species of *Gomphus* s.l. and species of *Ramaria* subgen. *Echinoramaria* (Giachini et al. 2010: figs. 1–2). Of the genera treated here, *Gomphus* s.s. and *Phaeoclavulina* share the most macro- and microscopic features. Although *Phaeoclavulina* is mostly ramarioid, it also includes a few pileate species previously placed in *Gomphus* s.l. Both *Gomphus* s.s. and *Phaeoclavulina* have uni- and multi-pileate species that

present fan- to funnel-shaped, glabrous to subtomentose or floccose-squamose pilei. Nearly all species have decurrent hymenia with wrinkled hymenial folds, and all species possess clamp connections.

Gomphus s.s. and *Gloeocantharellus* are fairly easily differentiated by abundance of gleoplerous hyphae and clamp connections: *Gomphus* s.s. has ubiquitous clamp connections, whereas these structures are present only irregularly in *Gloeocantharellus*. *Gloeocantharellus* is characterized by abundant gleoplerous hyphae dispersed among all hyphal tissues, separating it from other genera in the *Gomphaceae*.

Turbinellus was proposed by Earle (1909) to accommodate *Cantharellus floccosus*. Earle (1909) assigned three species to his genus, all from North America. *Turbinellus* species were later transferred to *Gomphus* (Singer 1945), then *Cantharellus* (Smith & Morse 1947), *Nevrophyllum* (Heim 1954: only *T. floccosus*), and finally back to *Gomphus* (Corner 1966). The taxonomy of its species has been challenging, primarily to the lack of morphological features sufficiently reliable to differentiate species, resulting in many nomenclatural synonyms.

The morphological and genetic sampling of all species, forms, and subspecies by Giachini (2004), Giachini et al. (2010), and this study support the new generic circumscriptions reflected here. Many species previously delimited on morphological grounds have been shown to represent phenotypic variants of a few well-supported genetic taxa. Genetic data indicate that *Gomphus bonarii* and *G. floccosus* (and all their forms and subspecies) represent phenotypes of a single species (Giachini et al. 2010). Hence, *G. bonarii* and *G. floccosus* (and all their variants), long thought to be different species but seldom correctly separated on morphological grounds, are synonymized, with the name *Turbinellus floccosus* having priority. Even though Giachini (2004: fig. 3) presented three clades of *bonarii/floccosus* species, the overall morphological similarities (e.g., basidioma morphology, spore size, hyphal arrangement) combined with the inconsistent arrangement of species/subspecies within and among each of those clades, imply that those species/subspecies are sufficiently similar to comprise one single taxon.

The overall conclusions indicate an intricate organization for *Gomphus* s.l. and its counterparts. Additional studies, focusing on generic and species distribution, are undoubtedly necessary.

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New records of *Uredinales* from Fairy Meadows, Pakistan

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ABSTRACT — *Puccinia pygmaea* var. *angusta* and *P. urticata* var. *urticata* were collected in Fairy Meadows and are new records for Pakistan. Similarly the aecidial stages of *Uromyces hedysari-obscuri* and *U. polygoni-avicularis* are an addition to the rust flora of this country. *Puccinia alpina, P. leveillei,* and *P. ribis* are redescribed and illustrated from Fairy Meadows.

KEY WORDS — *Bistorta vivipara*, Nanga Parbat

Introduction

Fairy Meadows in Northern Pakistan is a floristically rich area, from which about 70 species of rust fungi have been reported (Afshan et al. 2009). During several extensive surveys of the region, seven plants infected with rust fungi were collected. Among these, *Puccinia pygmaea* var. *angusta* on *Calamagrostis epigejos* and *P. urticata* var. *urticata* on *Carex flacca* are new records for Pakistan. The aecidial stages (I) of *Uromyces hedysari-obscuri* and *U. polygoni-avicularis* are also here described as an addition to the rust flora of Pakistan. *Puccinia alpina, P. leveillei,* and *P. ribis,* which represent new records for Fairy Meadows, are re-described to illustrate important morphological features using scanning electron microscopy.

Materials & methods

Freehand sections of infected tissue and spores were mounted in lactophenol and gently heated to boiling. The preparations were observed under a NIKON YS 100 microscope and photographed with a digipro-Labomed and JSM5910 scanning electron microscope. Drawings of spores and paraphyses were made using a Camera Lucida (Ernst Leitz Wetzlar, Germany). Spore dimensions were taken by an ocular micrometer. At least 25 spores were measured for each spore stage. Images were also obtained of the rust spores using a scanning electron microscope (SEM). The rusted specimens have been deposited in the herbarium of the Botany Department at the University of the Punjab, Lahore (LAH).

Taxonomy

Puccinia pygmaea* var. *angusta Cummins & H.C. Greene, Mycologia 58: 715 (1966). FIG. 1

SPERMOGONIA and AECIA not found. UREDINIA amphigenous, mostly on adaxial surface, pale brown to golden brown, 0.09–0.3 × 0.1–0.4 mm. UREDINIOSPORES ellipsoid to broadly ellipsoid or ovoid, 21–25 × 25–29 µm; wall 1.5–2 µm thick, pale yellow to pale brown, echinulate; germ pores obscure, 4–6(–8), scattered. PARAPHYSES clavate to capitate, peripheral to intermixed, hyaline to pale brown, 14–18 µm wide, to 80 µm long. TELIA amphigenous, covered by epidermis, dark brown to blackish brown, loculate, 0.09–0.4 × 0.2–0.6 mm. TELIOSPORES 1–2 celled, clavate or ellipsoid; wall 1.5–2 µm thick, cinnamon brown to chestnut brown, smooth; 15–23 × 42–58 µm (mean 16.6 × 47.5 µm); apex mostly truncate, sometimes rounded to conical, 4–6(–7) µm thick; germ pore obscure; pedicel hyaline to pale brown, 5–7 × 8–15 µm, one-celled spores abundant.

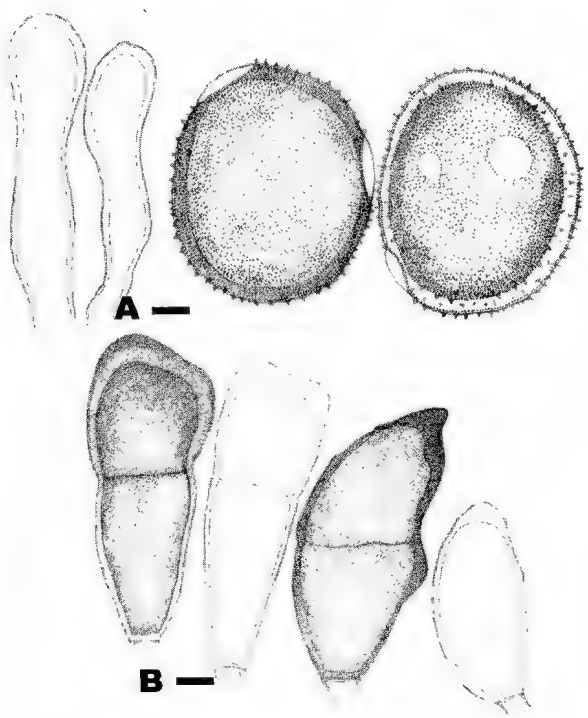

FIG. 1. Lucida drawings of *Puccinia pygmaea* var. *angusta*.
(A) Urediniospores and paraphyses. (B) 1 2 celled teliospores. Scale bar 10 µm.

MATERIAL EXAMINED: On *Calamagrostis epigejos* (L.) Roth (*Poaceae*), with II and III stages, Pakistan, Northern Areas, Fairy Meadows, 3036 m, 12 Aug 2007, N.S.Afshan #G 90. (LAH Herbarium NSA 1083).

COMMENTS: Six varieties of *P. pygmaea* are recognized based on spore size: *P. pygmaea* var. *ammophilina* (Mains) Cummins & H.C. Greene, *P. pygmaea* var. *angusta*, *P. pygmaea* var. *chisosana* Cummins, *P. pygmaea* var. *major* Cummins & H.C. Greene, *P. pygmaea* var. *minor* Cummins & H.C. Greene, and *P. pygmaea* Eriksss. var. *pygmaea* (Cummins 1971).

Ahmad (1956a, b) reported *P. pygmaea* on *Agrostis munroana* Aitch. & Hemsl. from Kaghan valley, and Afshan & Khalid (2009) reported *P. pygmaea* var. *pygmaea* on *Agrostis gigantea* Roth from Bara Gali (NWFP). The variety *P. pygmaea* var. *angusta* is a new record for Pakistan.

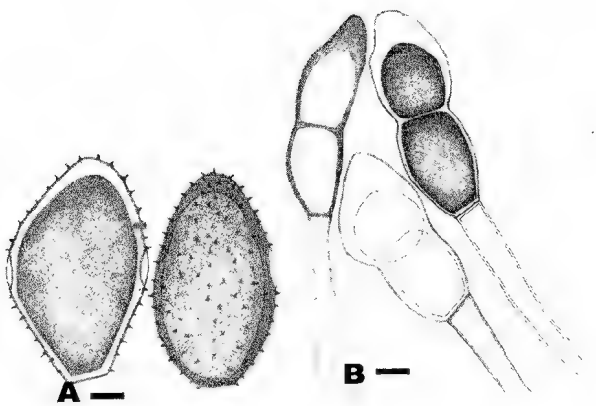

FIG. 2. Lucida drawings of *Puccinia urticata* var. *urticata*. (A) Urediniospores. (B) Teliospores. Scale bar = 10 µm.

Puccinia urticata F. Kern, Mycologia 9: 214 (1917) var. ***urticata*** FIG. 2

SPERMOGONIA and AECIA unknown. UREDINIA mostly hypophyllous, sometimes amphigenous, on leaves and culms, intermixed with telia. UREDINIOSPORES ovoid to obovoid or ellipsoid to broadly ellipsoid, 18–24 × 23–31 µm (mean 22.0 × 27.6 µm); wall 1.5–2 µm thick, pale brown to cinnamon brown, finely and sparsely echinulate; germ pores 2–4, equatorial; pedicel hyaline, short. TELIA mostly hypophyllous, rarely amphigenous, intermixed with uredinia, dark brown to blackish brown, covered by epidermis, 0.1–0.3 × 0.2–0.5 mm. TELIOSPORES clavate to broadly clavate or oblong, not or slightly constricted at septum, 14–24 × (38–)42–71 µm (mean 19.2 × 52.5 µm); wall 2–3 µm thick, golden brown to chestnut brown, smooth; apex mostly rounded, 9–15 µm

thick; germ pores obscure; pedicel hyaline to pale brown, thin walled, 7–10 µm wide and up to 100 µm long.

MATERIAL EXAMINED: On *Carex flacca* Schreb. (*Cyperaceae*), with II and III stages, Pakistan, Northern Areas of Pakistan, Fairy meadows, 3036 m, 12 Aug 2007, N.S.Afshan #74. (LAH Herbarium NSA 1098).

Puccinia urticata var. *urticata* is a new record for Pakistan.

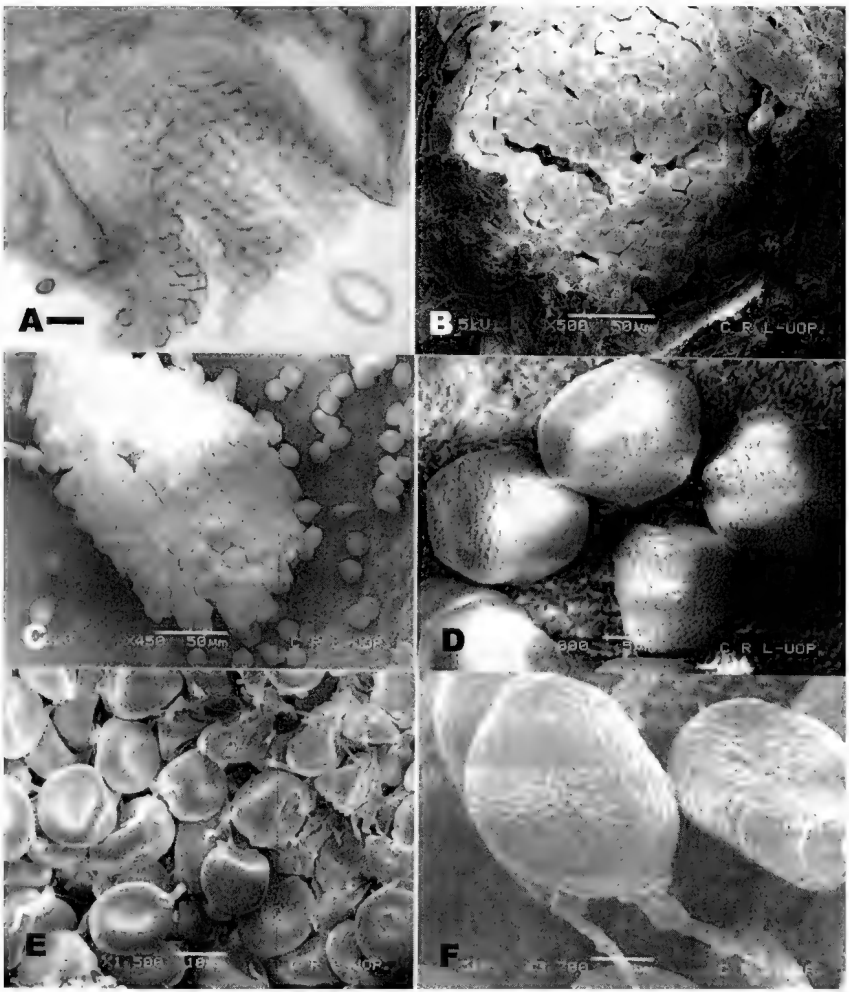

FIG. 3. *Uromyces hedysari-obscuri.* (A) Aecium containing aeciospores (B) SEM photograph of an aecium. (C) Aeciospores with peridial cells. (D) Aeciospores showing verrucose wall ornamentation. (E) SEM photograph of teliospores. (F) Teliospores showing verrucose wall ornamentation. Scale bar for E = 0.1 mm.

Uromyces hedysari-obscuri (DC.) Carestia & Picc., in Orbigny, Erb. critt. Ital., Ser. 2, 9: no. 447 (1871). FIGS. 3–4

SPERMOGONIA not known. AECIA mostly caulicolous, gregarious, in rounded groups. AECIOSPORES angular to globose, verrucose, hyaline with a yellowish tinge, 16–21 × 20–24 µm; peridial cells hyaline to pale yellow, angular to rectangular, verrucose, 20–24 × 23–31 µm. TELIA amphigenous, mostly epiphyllous, scattered or gregarious, small, roundish, early naked, surrounded by a ruptured epidermis, dark brown to blackish brown. TELIOSPORES globose, oblong or ellipsoid; 16–19 × 20–29 µm; apex rounded, with light colored papilla, 3–6 µm thick; wall 1.5–2.5 µm thick, dark brown to chestnut brown, verrucose; germ pores 2, equatorial; pedicel hyaline, short, deciduous, 6–7 × 14–16 µm.

MATERIAL EXAMINED: On *Hedysarum falconeri* Baker (*Fabaceae*), with I & III stages, Pakistan, Northern Areas, Fairy Meadows, 3036 m, 12^{h} Aug 2007. N.S.Afshan #G16. (LAH Herbarium NSA 1006).

The teliospore stage of *Uromyces hedysari-obscuri* has been reported on the same host from Fairy Meadows (Sultan 2005). The aecidial stage (I) of this fungus, which is described here for the first time from Pakistan, is an addition to our rust flora.

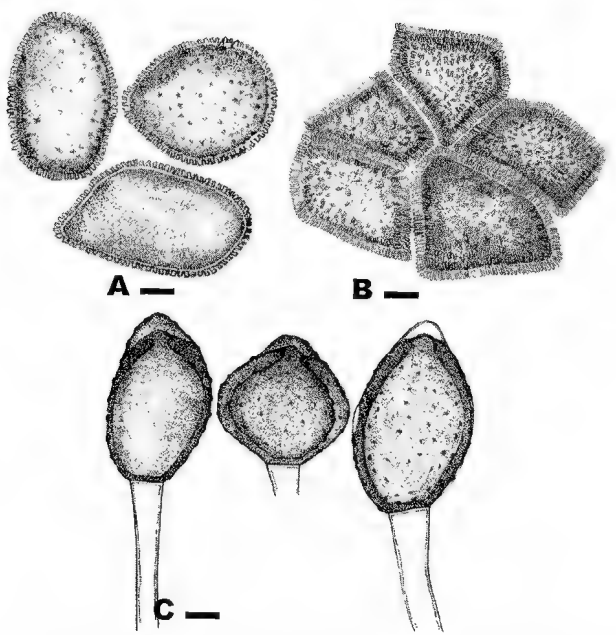

FIG. 4. Lucida drawings of *Uromyces hedysari-obscuri.*
(A) Aeciospores showing verrucose wall ornamentation. (B) Peridial cells.
(C) Teliospores showing verrucose wall ornamentation.
Scale bar for A 5 µm, B & C 10 µm.

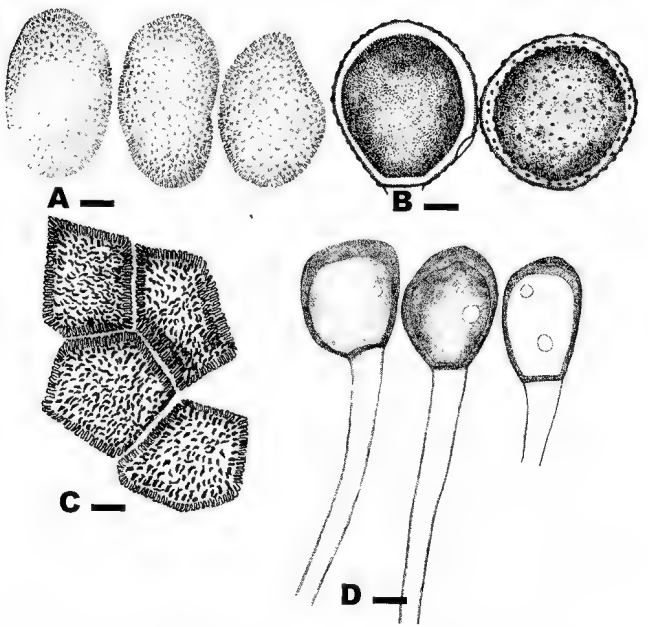

FIG. 5. Lucida drawings of *Uromyces polygoni-avicularis*.
(A) Peridial cells. (B) Aeciospores. (C) Urediniospores showing germ pores.
(D) Teliospores. Scale bar = 10 µm.

Uromyces polygoni-avicularis (Pers.) P. Karst., Bidr. Känn. Finl. Nat. Folk 4: 12 (1879), as '*polygoni avicularіae*'. FIGS. 5–6

AECIA hypophyllous, minute, in circular or irregular shaped groups, orange, cupulate. AECIOSPORES globose to ellipsoid, verrucose, hyaline to yellow with orange granules, 15–22 × 18–24 µm; peridial cells rhomboid, hyaline to pale yellow, striate, verrucose, 17–24 × 23–29 µm. UREDINIA amphigenous, scattered or gregarious, minute, roundish, brown to cinnamon brown, 0.09–0.3 × 0.2–0.8 mm. UREDINIOSPORES globose to subglobose or ellipsoid, 18–24 × 20–29 µm; wall 1.5–3.0 µm thick, golden brown to cinnamon brown, verruculose; germ pores 2–4, equatorial. TELIA amphigenous, small, scattered or gregarious, on leaves and stem, roundish or oblong, blackish brown, 0.1–0.5 × 0.2–0.8 mm. TELIOSPORES subglobose, ovate or ellipsoid, 18–24 × 22–31(–35) µm; apex rounded or truncate, 4–6 µm thick; wall 2–3 µm thick, smooth, golden brown to chestnut brown; germ pore, apical or sub-apical; pedicel hyaline to pale yellow, persistent, 8–10 × 90–104 µm.

MATERIAL EXAMINED: On *Bistorta vivipara* (L.) Delarbre (*Polygonaceae*), with II & III stages, Pakistan, Northern Areas, Bial camp (Nanga Parbat base camp), 11 Aug 2007. N.S.Afshan #G10. (LAH Herbarium NSA 1008). On *Polygonum aviculare* L.

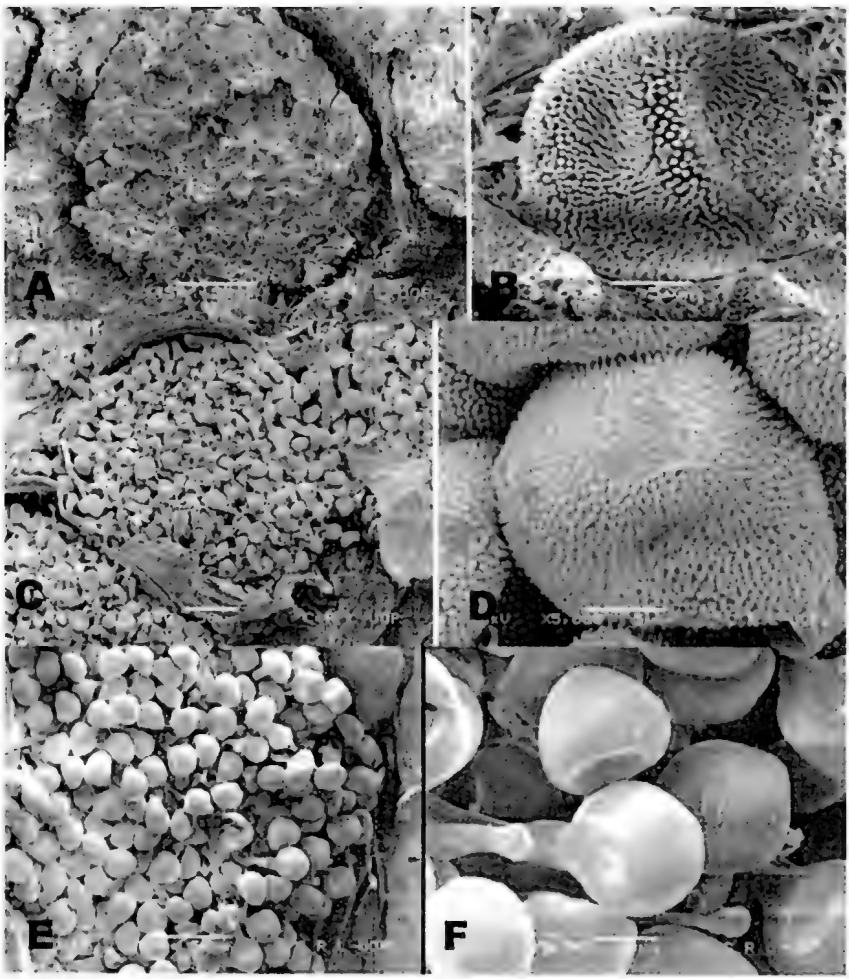

FIG. 6. *Uromyces polygoni-avicularis*. (A) SEM photograph of an aecium. (B) An aeciospore showing verrucose wall ornamentation. (C) SEM photograph of a uredinial sorus. (D) SEM photograph of a urediniospore showing verrucose wall ornamentation. (E) SEM photograph of a telium containing teliospores. (F) Teliospores.

(*Polygonaceae*), with I & III stages, Pakistan, Northern Areas, Fairy Meadows, 3036 m, 12 Aug 2007. N.S.Afshan #G02. (LAH Herbarium NSA 1007)

The uredinial and telial stages of *Uromyces polygoni-avicularis* were previously reported on *Polygonum aviculare* from Kaghan and Quetta valleys and on *Bistorta vivipara* (as *P. viviparum* L.) from Hazara, Madian, and Swat (Ahmad et al. 1997). The aecidial stage represents a new record for Pakistan

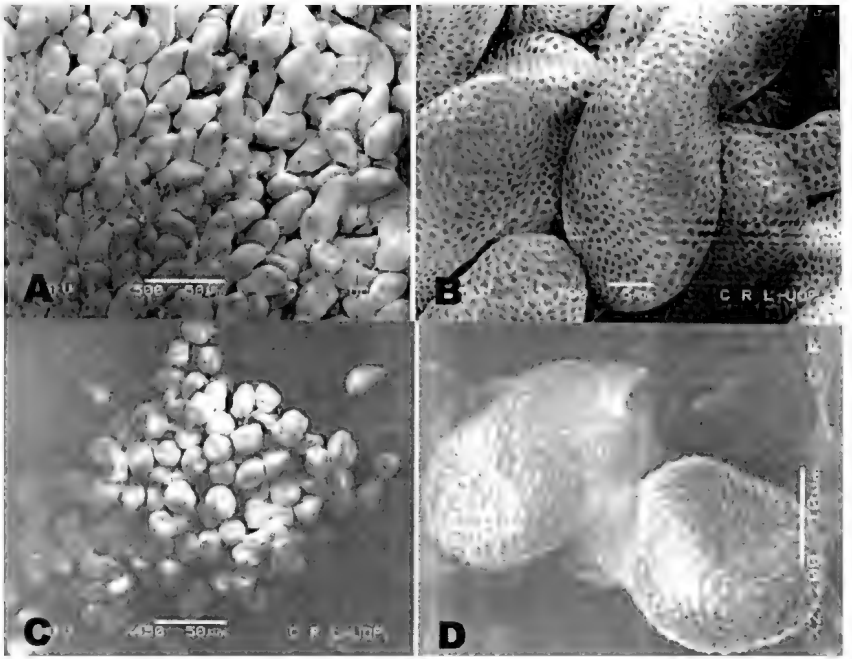

FIG. 7. A B. *Puccinia alpina.* (A) SEM photograph of telium showing teliospores. (B) Teliospores showing striately verrucose wall ornamentation. C D: *Puccinia leveillei.* (C) SEM photograph of a telium (D) Teliospores showing verrucose wall ornamentation.

Puccinia alpina Fuckel, Jahrb. Nassauischen Vereins Naturk. 27–28: 13 (1873).
FIG. 7A–B, 8A

SPERMOGONIA, AECIA and UREDINIA unknown. TELIA hypophyllous, scattered or loosely clustered, small roundish, soon naked, pulvinate, dark brown to blackish brown, 0.08–0.1 × 0.2–0.8 mm. TELIOSPORES ellipsoid to oblong or clavate, rounded at both ends or sometimes attenuated downwards, not or slightly constricted at septum, base roundish or narrowed, 16–26 × (25–)31–50 µm; wall 1.5–2 µm thick, verrucose, golden brown to chestnut brown; apex 3–5 µm thick, pale in color; germ pore 1 per cell, germ pore of both cells near septum or sometimes apical cell, with hyaline umbo over each pore; pedicel hyaline, deciduous, up to 25 µm long and 5–6 µm wide.

MATERIAL EXAMINED: On *Viola biflora* L. (*Violaceae*), with III stage, Pakistan, Northern Areas, Fairy Meadows, 3036 m, 12 Aug 2007. N.S.Afshan #G 08. (LAH Herbarium NSA 1005).

Ono (1992) previously reported *Puccinia alpina* on *Viola rupestris* F.W. Schmidt from Kaghan valley. The above collection is a new record for Fairy Meadows.

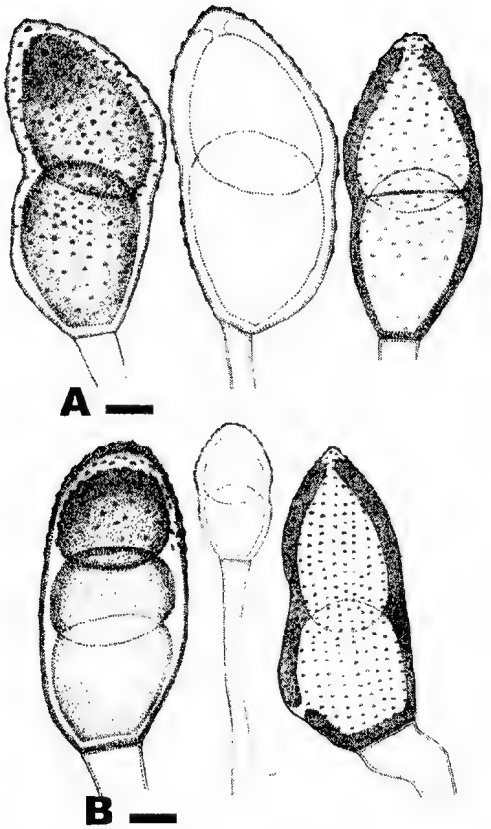

FIG. 8. (A) Lucida drawing of teliospores of *Puccinia alpina*.
(B) Lucida drawing of teliospores of *Puccinia leveillei*.
Scale bars 10 µm.

Puccinia leveillei Mont., in Gay, Hist. Fis. Polit. Chile 8: 41 (1852). FIG. 7C–D, 8B
P. geranii-sylvatici P. Karst., Enum. Fung. Lapponia: 220 (1866).

SPERMOGONIA, AECIA and UREDINIA unknown. TELIA mostly amphigenous, 0.2–0.4 × 0.2–0.6 mm, dark brown. TELIOSPORES mostly two-celled, sometimes three-celled; ellipsoid to clavate, not or slightly constricted at septa, 14–23 × 30–44(–47) µm; wall 2–3 µm thick, densely verrucose, brown to chestnut brown; apex rounded or truncate, 4–5 µm thick; germ pore of upper cell mostly apical, sometimes sub-apical, of lower cell near pedicel; pedicel short, hyaline, 8–9 × 10–15 µm.

MATERIAL EXAMINED: On *Geranium sibiricum* L. (*Geraniaceae*), with III stage, Pakistan, Northern Areas, Fairy Meadows, Bial camp (Nanga Parbat base camp) 3036 m, 12 Aug 2007. N.S.Afshan # G13. (LAH Herbarium NSA 1010).

Ahmad 1956a reported *P. leveillei* on *Geranium pratense* L., *G. collinum* Stephan ex Willd., *G. rectum* Trautv., and *G. eglandulosum* Dalla Torre from Kaghan, Kalam, Baltistan, and Barum valleys. This above collection represents a new record from Fairy Meadows (Bial camp); *Geranium sibiricum* is a new host species in Pakistan.

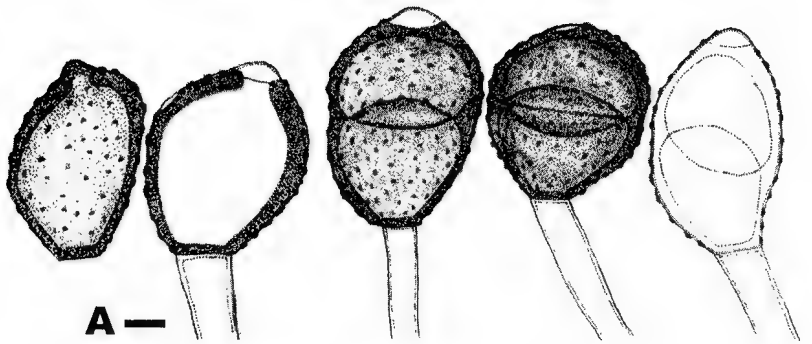

Fig. 9. Lucida drawing of *Puccinia ribis*.
1 2 celled teliospores showing verrucose wall ornamentation. Scale bar = 10 µm.

Puccinia ribis DC., Fl. franç., 3e Ed., 2: 221 (1805). Fig. 9

Spermogonia unknown, aecia and uredinia absent. Telia epiphyllous, surrounded by a discolored yellow zone, circinate, pulverulent, 0.9–3 × 3–5 mm, chestnut brown. Teliospores ovoid or oblong to ellipsoid, rounded above and below, hardly constricted, 15–24 × 20–35 µm; wall 1.5–2.5 µm thick at sides and 4–5 µm thick at apex, verrucose, chestnut brown; germ pores 1 per cell, apical in upper cell with a hyaline papilla, pore of lower cell usually basal; pedicels hyaline, deciduous, 4–5 × 18–25 µm. A few one-celled spores also observed.

Material examined: On *Ribes nigrum* L. (*Grossulariaceae*), with III stage, Pakistan, Northern Areas, Fairy Meadows, 3036 m, 12th Aug 2007. N.S. Afshan # G14. (LAH Herbarium NSA 1011).

Ahmad 1956a reported *P. ribis* on *Ribes rubrum* L. from Swat, Kalam, and Hazara. Our collection represents a new record from Fairy Meadows.

Acknowledgements

We sincerely thank Dr. Amy Rossman, Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, and Omar Paíno Perdomo, Dominican Mycological Society, Santo Domingo, for their valuable suggestions to improve the manuscript and acting as presubmission reviewers. We are thankful to Dr. Asim Sultan for help in the field. We are highly obliged to Higher Education Commission (HEC) of Pakistan for providing financial support.

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Observations on *Melanoleuca*. Type studies – 3

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Abstract — The results of type studies on several taxa of the genus *Melanoleuca* are reported and discussed: *Melanoleuca substrictipes*, *M. polioleuca*, *M. alutaceopallens*, *M. permixta*, *M. wrightii*, *M. lapataiae*, and *M. lapataiae* var. *ochroleuca*. For the first two taxa a lectotype and a neotype are designated respectively.

Key words — taxonomy, *Tricholomatales*

Introduction

Type materials of sixteen critical *Melanoleuca* taxa have already been discussed in two previous publications (Fontenla & Para 2007, 2008). Fontenla & Para (2007) reported on *M. diverticulata* G. Moreno & Bon, *M. electropoda* Maire & Malençon, *M. kavinae* (Pilát & Veselý) Singer, *M. meridionalis* G. Moreno & Barrasa, *M. metrodii* Bon, *M. nigrescens* (Bres.) Bon, and *M. pseudobrevipes* Bon. Fontenla & Para (2008) discussed *Melanoleuca decembris* Métrod ex Bon, *Tricholoma humile* var. *bulbosum* Peck, *M. luteolosperma* (Britzelm.) Singer, *Clitocybe nobilis* Peck, *M. pseudopaedida* Bon, *M. decembris* var. *pseudorasilis* Bon, *M. subalpina* (Britzelm.) Bresinsky & Stangl, *M. subdura* (Banning & Peck) Murrill, and *M. tucumanensis* Singer.

In this paper we hope to shed new light on seven more taxonomically critical taxa and designate one lectotype and two neotypes to clarify relationships of the three taxa to their closest allies.

Materials & methods

Microscopical characters were examined using a Leitz Biomed optical microscope equipped with 100×, 500× and 1000× lenses; a stereomicroscope mod. MbC-10 was used to make thin sections. The pileipellis was sectioned from radial sections of the pileus margins, and the stipitipellis was sectioned from longitudinal sections of the medial third of the stipe. Sections were routinely revived in L4, stained in Phloxine B,

and tested for amyloidity in Melzer's. Micrographs were made using a Nikon Coolpix 4500 digital camera. Throughout all text, citations in quotes ("...") comes from the original protologues (even if incorrect), except that the en-dash (–) is substituted to separate data.

The systematic framework adopted follows Bon (1991) with some personal changes.

Taxonomy

Melanoleuca substrictipes Kühner, Bull. Soc. Linn. Lyon 47(1): 52. 1978. FIG. 1

ORIGINAL DIAGNOSIS: *Pileus 25 105 mm latus, carnosulus, e convexo expansus, saepe obtuse umbonatus, jove pluvio albus vel eburneolo albus, sed mox albus, demum saepe alutaceus vel subisabellinus. Stipes 17 80 mm altus, 4 14 mm crassus, subaequalis, albus, glaber, apice subtiliter pruinosus, solidus vel farctus. Lamellae confertae, plus minusve sinuatae vel emarginatae, albidae. Odore spermatico. Sporae 8 10 × 5 6.5 µm, ellipsoideae, modice elongatae, asperulae. Cystidiae fusoideae-subcuspidatae, angustae, inferne 5 9 µm, superne 2 3 µm latae, septatae. In pascuis alpinis, frequens. Typus Herb. Kühner, n° 66-13.*

SPECIMEN STUDIED "Herbier R. Kühner (G) *Melanoleuca substrictipes* R. Kühner F Savoie Parc Nat. de la Vanoise, environs de Pralognan Entre le Pas de L'Ane et le Cirque des Nants Alt.: 1900 « K 66-13 » Leg./Det.: R. Kühner 20-07-1966" **lectotypus, hic designatus.** [G, Conservatoire et Jardin botaniques de la ville de Genève, GENEVA, Switzerland]. Revision date: 26 August 2008.

(MACROCHARACTERS, see Kühner 1978: 46–49).

MICROCHARACTERS —Basidiospores 7.2–9.6 × 4.8–6.0 µm; av. = 8.60 × 5.55 µm; Q = 1.35–1.75; Qm = 1.55; ellipsoid, with small, dense, isolate and rounded warts, without a suprahilar plage. Basidia 31–33 × 7–8 µm, clavate, 4-spored. Cheilocystidia 45–55 × 6–8 µm, rare, urticiform, with a thin wall, hyaline, sometimes with incrusted apex. Pleurocystidia not seen. Stipitipellis made up of long, narrow hyphae, intermixed with shorter ones constricted at septa, with scattered short, clavate or cylindroid cells, not in clusters; caulocystidia not seen. Pileipellis a cutis of interwoven hyphae, without emergent elements, with a thin gelatinized suprapellis. Dermatocystidia and thromboplerous hyphae absent. Clamp connections absent.

COMMENTS - The authentic material kept in Geneva (G) is subdivided into two portions. "Pr. 66-13" is composed of fragments of several pilei and stipes and is difficult to study; "66-13 lot B" consists of basidiomata sections, all in good state. Each part is accompanied by various barely legible hand-written notes, interpreted for us by Pierre Arthur Moreau: Envelope 66-13 (FIG. 1) — "*Melanoleuca* Pr.66-13 (Pr is for Pralognan, the name of the locality in the Vanoise National Park), 20 July 1966, 17 carpophores (remarquable qualité !) Odeur vite aigrelette bolétoïde." Envelope 66-13 lot B — "Melanoleuca Pr 66.13, lot B, 3 exemplaires. 20 July 1966. Sûrement pas spermatique en coupe!"

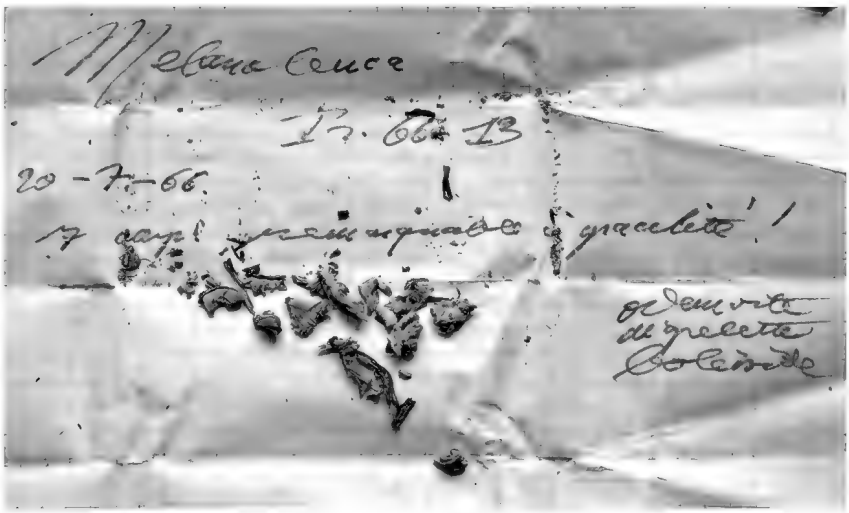

FIGURE 1. *Melanoleuca substrictipes*. 66-13 envelope (lectotypus).

However, it seems clear that the two parts represent two different collections made the same day – 20 July 1966 – and that Kühner decided to separate them due to differences in appearance and odor. In Pr. 66-13, we observed urticoid (fusoid) cystidia, as reported in the protologue, while in 66-13 "lot B", we noted numerous fusoid macrocystidia, very different from those in the Pr. 66–13 basidiomata.

The indication of the "aigrelette boletoide" odor for Pr. 66-13 matches the protologue description (Kühner 1978: 370), while the "lot B" spermatic odor ("sûrement pas spermatique") contradicts "souvent distinctement spermatique après la coupure" noted in the protologue.

Based on ICBN Arts. 9.9 and 9.12 (McNeill et al. 2006), we here designate the portion of the original material marked Pr. 66-13 as lectotype of *M. substrictipes*, since the protologue is also based on this material and it fits the protologue very well.

Melanoleuca favrei Bon is a closely related species with longer spores (Qm = 1.71, as confirmed by our type study).

Other similar European species are *M. substrictipes* var. *sarcophylla* Kühner and *M. pseudoevenosa* Bon ex Bon & G. Moreno, which have ochraceous gills at maturity and spore ratios of Qm = 1.56 and 1.72, respectively. Comparable extraeuropean species are *M. conspurcata* (Berk. & Broome) Pegler and *M. tropicalis* Pegler, which our type studies showed to have spore ratios of Qm = 1.48 and 1.65, respectively. We need more data to confirm whether these differences are taxonomically significant.

Melanoleuca alutaceopallens (P. Karst.) Konrad & Maubl., Icon. Select. Fung. 6(10): 324. 1937.

≡ *Tricholoma melaleucum var. alutaceopallens* P. Karst., Medd. Soc. F. Fl. Fenn. 9: 40. 1882.

≡ *Tricholoma melaleucum* subsp. *alutaceopallens* (P. Karst.) Sacc.., Syll. Fung. 5: 135. 1887.

≡ *Tricholoma alutaceopallens* (P. Karst.) P. Karst., Medd. Soc. F. Fl. Fenn. 16: 85. 1890 ("1889").

ORIGINAL DIAGNOSIS: *Pileus carnosus, mollis, e convexo planus, obsolete umbonatus, laevis, glaber, alutaceopallens, vix hygrophanus, circ. 7 cm. latus. Stipes farctus, rigidofragilis, strictus, aequalis, basi incrassatus, primitus squamuloso-fibrillosus, glabrescens, apice pruinosus, albidus, demum nigrescens, 6–7 cm. longus, 4–5 mm. crassus. Lamellae emarginatae, planae, horizontales, rectae, lineares, sat angustae, admodum confertae, integerrimae, albae, 4–5 mm. latae. Caro alba, mollis, non hygrophana. In clivis sterilibus prope Mustiala, m. Sept. 1882.*

SPECIMEN STUDIED: "Museum Botanicum Universitatis, Helsinki (H) Herbarium Petter Adolf Karsten (1834–1917) #5270 (*Tricholoma*) n-sp. *Tricholoma melaleucum* (Pers.) var. FINLAND. ETELÄ-HÄME (EH/Ta). Tammela. Mustiala Grid 27° E: [no date] leg. & det. P.A. Karsten (H6003411)", **neotypus, hic designatus** (H). Revision date: 7 February 2008.

(MACROCHARACTERS, see Karsten 1882: 40).

MICROCHARACTERS – Basidiospores 7.8–10.8 × 4.8–6.2 µm; av. = 8.82 × 5.73 µm; Q = 1.25–2.00; Qm = 1.55; subglobose or subcylindrical, with small or medium, weakly amyloid warts, which are dense, isolate, rounded, rarely, with well-delimited suprahilar plage. Basidia 23–30 × 7–10 µm, clavate, 4-spored. Cheilocystidia numerous, macrocystidia type, 65–85 × 12–19 µm, lageniform or fusoid in shape, thin-walled, with crystals at apex. Pleurocystidia scarce, similar to cheilocystidia. Stipitipellis made up of hyphae up to 240 × 20 µm, without emergent elements or caulocystidia. Pileipellis made up by interwoven hyphae, with conspicuous yellow-tawny intracellular pigment. Dermatocystidia and thromboplerous hyphae absent. Clamp connections absent.

COMMENTS – The type collection is composed of three entire basidiomata in good condition. No collection date is noted on the specimen envelope, but the locality, which is included, agrees with the original diagnosis ("prope Mustiala"). Karsten's envelope bears the herbarium number "5270", but the material is filed at H with the number H6003411. As we cannot assume that Karsten's original description is based on H6003411, it cannot be selected as a lectotype. However, given the lack of other material suitable for a lectotype selection, we think that the above collection can be designated as the neotype of *Tricholoma melaleucum* var. *alutaceopallens*.

This species is difficult to separate from other representatives of the same subgenus, which seem to differ mainly on macroscopical features that are lost in drying (pileus colour etc.): e.g., *M. melaleuca* (Pers.) Murrill, *M. cavipes* Métrod ex Bon, *M. pallidicutis* Bresinsky.

Karsten (1890) also described *Tricholoma alutaceopallens* var. *stercorarium*, which has not yet been transferred to *Melanoleuca* and which differs from the typical form (var. *alutaceopallens*) in darker pileus color and growth on dung.

Melanoleuca polioleuca (Fr.) Kühner & Maire, Bull. trimest. Soc. mycol. Fr. 50: 18 (1934).

≡ *Agaricus melaleucus* var. *polioleucus* Fr., Syst. Myc. 1: 115. 1821.

≡ *Agaricus polioleucus* (Fr.) Pass., Nuovo Giorn. Bot. Ital. 4: 58. 1872.

≡ *Tricholoma melaleucum* var. *polioleucum* (Fr.) Gillet, Les Hyménomycètes: 128. 1874.

≡ *Tricholoma melaleucum* subsp. *polioleucum* (Fr.) Sacc., Syll. Fung. 5: 134. 1887.

≡ *Tricholoma polioleucum* (Fr.) Lapl., Dict. Iconogr. Champ. Sup.: 339. 1894.

≡ *Melanoleuca vulgaris* var. *polioleuca* (Fr.) Konrad & Maubl., Icon. Select. Fung.6(10): 322. 1937.

≡ *Melanoleuca melaleuca* var. *polioleuca* (Fr.) J. Favre, Ergebn. wiss.Unters. schweiz. Nat. Parks 6(42): 441. 1960, comb. inval. (basionym lacking).

ORIGINAL DIAGNOSIS: *A.* melaleucus γ. polioleucus, *pileo griseo-livido, lamellis sordide albis, stipite subfarcto brevi, apice albo-pruinato. Constanter utique ab α. diversus nec elegans; sed e solo locoque diverso ortum puto; plurima enim utrique specei communia. Stipes 2 unc. longus, 2 3 lin. crassus, æqualis, sat firmus. Pileus obtuse umbonatus. Lam. latæ. Accedit ad* A. oreinum, *ut* A. melaleuc. *ad* A. humilem. *In regionibus depressis, campestribus, locis graminosis. (Lund, Alnarp etc) Oct. Nov. (v.v.)*

SPECIMEN STUDIED: "Mus. Botan. Stockholm. – Fungi suecici – *Tricholoma polioleuca* Småland: Femsjö s:n, Femsjö, Hägnen, Grytskedsängen, åker. 4.8.48 – G. Haglund et Seth Lundell (S) reg. nr. F70162", **neotypus, hic designatus** (S). Revision date: 1 October 2008.

(MACROCHARACTERS, see Fries 1821: 115).

MICROCHARACTERS – Basidiospores 6.0–8.4 × 3.8–4.8 µm; av. = 7.24 × 4.66; Q = 1.30–1.88; Qm = 1.56; with small, dense, amyloid warts, which are isolate and rounded, more rarely elongated, with well-delimited suprahilar plage. Basidia 28–33 × 8.5–9.5 µm, clavate, 4-spored. Cheilocystidia 50–70 × 12–17 µm, frequent, mostly fusiform, thin-walled. Stipitipellis made up of long, narrow hyphae, with scattered, small clusters of short, clavate cells, intermixed with caulocystidia, which are similar to cheilocystidia. Pileipellis made up of a thick ixocutis. Dermatocystidia and thromboplerous hyphae absent. Clamp connections absent.

COMMENTS – The type collection comprises four entire basidiomata in good condition. These specimens were collected in the same place where Fries used to collect and its micromorphological features match those proposed by nearly all authors as characterizing the taxon "*polioleucus*" (see Métrod 1949, Singer & Clémençon 1972, Bresinsky & Stangl 1977, Kühner 1978, Enderle & Krieglsteiner 1987, Bon 1991, Gulden 1992, Moreno et al. 1994, Lonati 1998, Watling & Turnbull 1998, Boekhout 1999, Grilli & Lanzoni 1999, Fontenla et al. 2001, Munzmay 2005, Caroti et al. 2006, Vesterholt 2008). Therefore, since

there is no original material available, this collection is here proposed as the neotype of *Agaricus melaleucus* var. *polioleucus*.

Melanoleuca melaleuca has white flesh, a non-pruinose stipe, and usually grows in the forests; *M. subpulverulenta* (Pers.) Singer has lageniform cystidia, a grey pileus, and grows in the forests; *M. heterocystidiosa* (Bon & Beller) Bon has grey pileus and fusiform or lageniform cystidia; *M. polioleuca* f. *langei* Boekhout differs from the typical form only in the relatively short stipe; *M. polioleuca* f. *pusilla* Boekhout & Kuyper is smaller and grows in grassland; and *M. polioleuca* var. *confusa* G. Moreno has pale stipe flesh and grows under coniferous trees.

Neotypes should be designated for the last two taxa, as their holotypes are believed to be missing (personal communication by L and AH herbaria curators).

Melanoleuca permixta Raithelh., Hong. Argentin. 1: 139. 1974. FIG. 2

ORIGINAL DIAGNOSIS: *Pileo sordidae pallido, centro fusco, convexo-umbonato, glabro, 4–5 cm lato. Odore grato, sapore miti. Lamellis cremeis, sinuatis(-adnatis), subdistantibus. Stipite pallido, cavo, 4–5 cm/6–8 mm, carne pallida. Sporis in cumulo candidis, amyloides, subverrucosis, 7.5–9/4.5–6 my. Cheilocystidiis nullis. Pratis, autumno.*

Typus: Veronica/Prov. Buenos Aires número MeA-10472 in herb. mihi conservatur.

SPECIMEN STUDIED: "*M. permixta*, nr. 104-72", holotype (Z+ZT). Revision date: 3 September 2008.

(MACROCHARACTERS, see Raithelhuber 1974: 139).

MICROCHARACTERS – Basidiospores 8.2–9.8 × 5.0–6.2 µm; av. = 8.58 × 5.77 µm; Q = 1.35–1.78; Qm = 1.49; with thick, dense, isolate, amyloid warts, rounded or more rarely elongated in shape, with well-delimited suprahilar plage. Basidia 28–31 × 7–8 µm, clavate, 4-spored or 2-spored. Cheilocystidia 37–55 × 7–10 µm, frequent, urticoid (fusoid-shaped), thin-walled, often with crystals at the apex. Pleurocystidia similar to cheilocystidia, frequent, especially in the sinus of lamellae. Stipitipellis made up of long, narrow hyphae, with numerous clusters of short, clavate or cylindraceous cells. caulocystidia not seen. Pileipellis made up of interwoven hyphae, with some ascending but not emergent elements. Dermatocystidia and thromboplerous hyphae absent. Clamp connections absent.

COMMENTS – The type collection is composed of two-thirds of a basidiome in good condition. In the protologue *Melanoleuca permixta* is described as an acystidiate species, but we found urticoid cystidia in the original material studied (FIG. 2), which would place this species in subgenus *Urticocystis*. This species is close to *M. exscissa* (Fr.) Singer owing to its colours and microscopic features, but the lack of further collections does not allow us to evaluate the variability of such features or to propose synonymy.

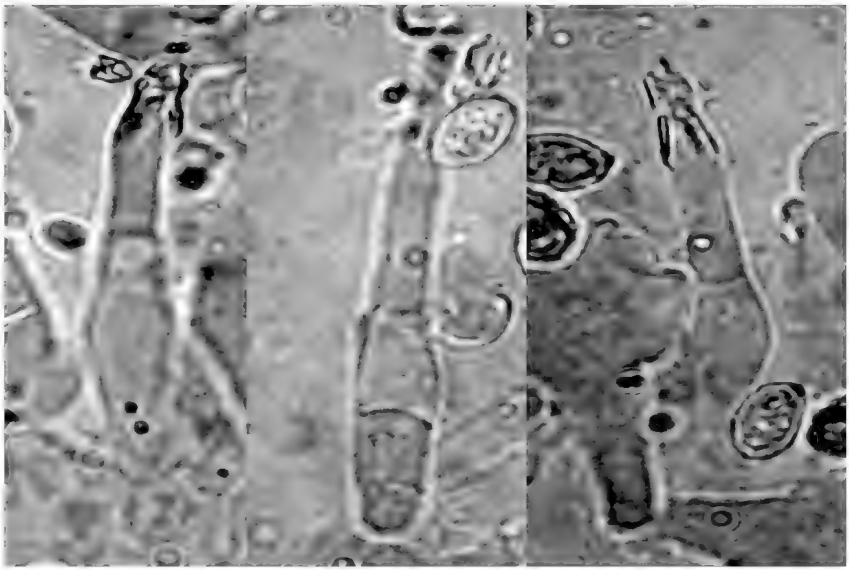

FIGURE 2. *Melanoleuca permixta*. Cystidia.

Melanoleuca wrightii Raithelh., Metrodiana 2(4): XXVII. 1971. FIG. 3

ORIGINAL DIAGNOSIS: *Pileo pallide griseo, centro suboscuriore, convexo depressove, gibboso, flexuoso, subviscoso, deinde nitente, 5 6.5 cm lato. Carne albida. Odore subingrato, sapore miti. Lamellis cremeis vel pallide griseis, basi clarioribus, 6 8 mm latis, adnatis vel dente decurrentibus, medio distantibus. Stipite cremeo, sursum suboscuriore; subfibrilloso, farcto, 2 3 cm longo, 5 7 mm lato. Sporis amyloideis, in cumulo albis, 8.5 11/6 6.5 my, subasperis. Ap.* Fraxinum *in hortis; autumno.*

Typus: Palermo/Buenos Aires. Sub numero MeA-3470 in herb. mihi conserv.

SPECIMEN STUDIED: "*M. wrightii*, nr. 3470", holotype (Z+ZT). Revision date: 3 September 2008.

(MACROCHARACTERS, see Raithelhuber 1971: XXVII).

MICROCHARACTERS – Basidiospores 7.4–9.6 × 4.8–6.2 µm; av. = 8.68 × 5.68 µm; Q = 1.20–1.80; Qm = 1.53; with thick, dense, isolate, amyloid warts, rounded or rarely elongated in shape, with well-delimited suprahilar plage. Basidia 33–38 × 8–9 µm, clavate, 4-spored or 2-spored. Cheilocystidia 35–50 × 8–10 µm, numerous, urticoid (typical in shape), thin-walled and with incrustated apex. Pleurocystidia very rare, similar to cheilocystidia. Stipitipellis with scattered, large clusters of short, clavate cells, intermixed with frequent urticoid (fusoid-shape) caulocystidia, 30–45 × 7–8 µm. Pileipellis made up of a trichoderm of ascendant cylindrical hyphae bearing apex-rounded terminal elements. Dermatocystidia and thromboplerous hyphae absent. Clamp connections absent.

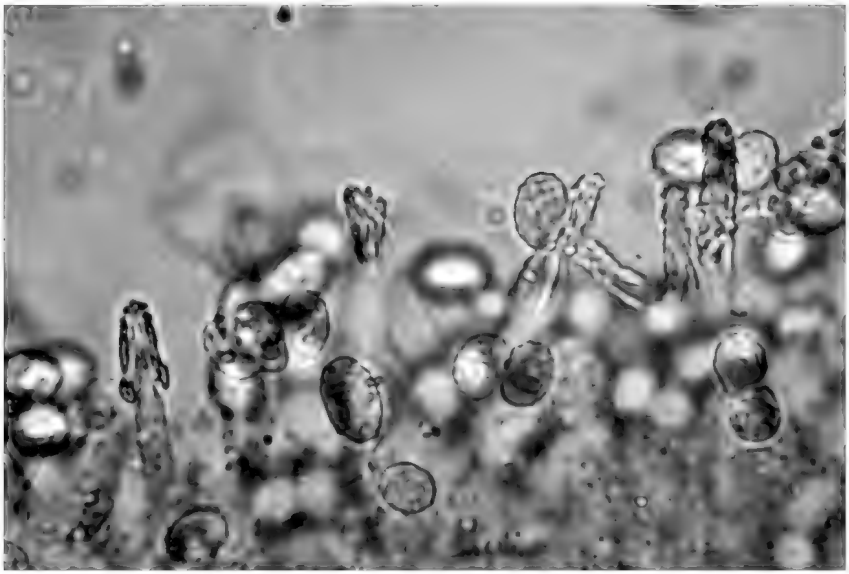

FIGURE 3. *Melanoleuca wrightii*. Cystidia.

COMMENTS – The type collection consists of one entire basidiome and one single pileus without stipe, all in good condition.

In the protologue the author does not record the presence of cystidia, but the original material exhibits typical cystidia (FIG. 3); accordingly, we place *M. wrightii* in subgenus *Urticocystis*. Like *M. permixta*, this species is close to *M. exscissa*, but the lack of further collections does not allow to estimate the variability of such features and to propose the synonymy.

Melanoleuca lapataiae Raithelh., Metrodiana 2(4): XXVI. 1971. FIG. 4

ORIGINAL DIAGNOSIS: *Pileo lilacino-griseo vel albido cum centro pallide lilacino-griseo, interdum maculoso, margine clariore, + gibboso, convexo, deinde depresso, glabro, vel partim nitido vel leviter tomentoso, interdum subsquamuloso; carne albida, sub cuticula + lilacina-grisea. 3 5 cm lato. Odore nullo, sapore miti. Lamellis albidis, nunc adnatis nunc dente decurrentibus nunc subliberis, saepe basi coniunctis. Stipite albido, interdum leviter grisaceo, primo farcto, adulto cavo, crustato, superficie leviter fibrilloso, rarior basi subbulboso, 3 4 cm longo, 4 10 mm lato; carne albida. Sporis amyloideis, in cumulo albidis vel candidis, asperis, 7 8.5/4.5 5.5 µm, ovoideis. Cheilocystidiis fibulisque nullis. Pascuis, partim cum Nothof. dombeyo.*

Typus: Lapataia/Argentina prope Hosteria Alakush. Sub numero MeA-271 in herb. mihi conserv.

SPECIMEN STUDIED: "*M. lapataiae Nr. 271*", holotype (Z+ZT). Revision date: 19 August 2008.

(Macrocharacters, see Raithelhuber 1971: xxvi–xxvii).

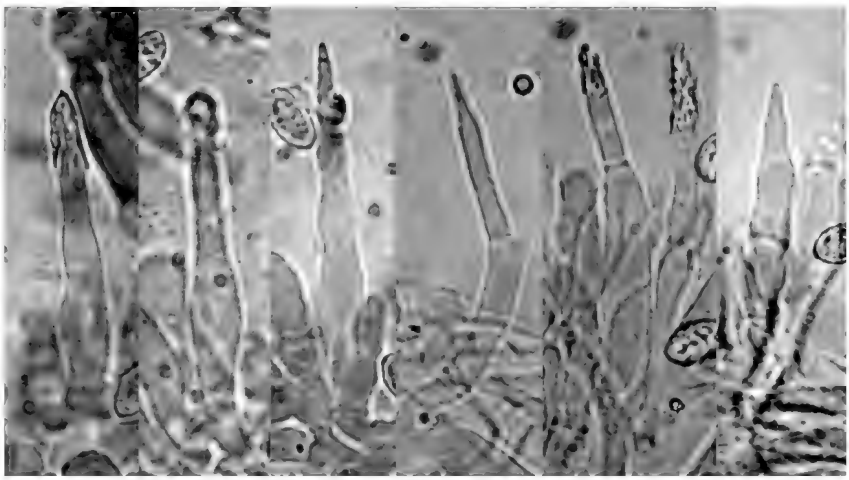

FIGURE 4. *Melanoleuca lapataiae*. Cystidia.

MICROCHARACTERS – Basidiospores 8.2–10.8 × 4.8–6 µm; av. = 9.48 × 5.79 µm; Q = 1.36–2.05; Qm = 1.64; with small, dense, isolate, rounded amyloid warts, with poorly developed but obvious suprahilar plage. Basidia 43–52 × 10–12 µm, clavate-elongate, 4-spored. Cheilocystidia 43–65 × 7–8 µm, abundant, macrocystidia type, fusoid, many of them bearing a septum, thin-walled, and with an encrusted apex. Pleurocystidia present but less numerous than cheilocystidia, even if very similar in size and shape. Stipitipellis made up of long, narrow hyphae, with frequent cylindrical cells, isolate or in clusters. caulocystidia not seen. Pileipellis made up of a trichoderm of ascendant hyphae with rounded and/or strangled terminal elements. Dermatocystidia and thromboplerous hyphae absent. Clamp connections absent.

COMMENTS – The type collection includes two entire basidiomata in good condition. Raithelhuber did not record the presence of cystidia for this species, either in the protologue (Raithelhuber 1971) or in two subsequent papers citing this agaric (Raithelhuber 1972, 1987). However, in a later description (Raithelhuber 1993), he wrote: "Cheilo (and pleuro?) cystidia 40–55/5–7 µm, fusiform, with thin wall."

These contradictions have so far made it impossible refer *M. lapataiae* to a subgenus. Study of the holotype (Z+ZT) allowed us to ascertain that *M. lapataiae* exhibits very unusually shaped cystidia (FIG. 4), which make it difficult to assign to any of the currently accepted cystidial types for *Melanoleuca*. We have never seen such cystidia in any of the very numerous collections of *Melanoleuca* we have examined. Due to its peculiar size and shape — always fusoid and mostly

septate — *M. lapataiae* takes an intermediate position between the subgenera *Urticocystis* and *Melanoleuca*. Only further studies, especially those based on a molecular approach, will provide further data on its taxonomic treatment.

Melanoleuca lapataiae var. ***ochroleuca*** Raithelh., Metrodiana Sonderheft 1: 16. 1972.

ORIGINAL DIAGNOSIS: *Differt M. lapataiae pileo albo-ochraceo vel cremeo, umbo + ochraceo, basi stipiti subaurantio-croceo in adultis et sporis 6.5 7.5 × 3.2 5 my.*
Typus: Lapataia, ripo Lago Rocae, sub numero MeA-371 in herb. mihi conserv.

SPECIMEN STUDIED: "*M. lapataiae* var. *ochroleuca, nr. 3-71*", holotype (Z+ZT). Revision date: 19 August 2008.

(MACROCHARACTERS, see Raithelhuber 1972: 16).

MICROCHARACTERS – Basidiospores 8.4–10.8 × 5.0–6.0 µm; av. = 9.21 × 5.67 µm; Q = 1.44–1.90; Qm = 1.63; with small to medium, dense, isolate, rounded, amyloid warts, with poorly developed but obvious suprahilar plage. Basidia 31–36 × 5–8 µm, clavate, 4-spored or 2-spored. Cheilocystidia 40–65 × 8–11 µm, not very frequent, macrocystidia type, fusoid, partially septate (in approximately 50% of cases), sometimes with encrusted apex, thin-walled, hyaline. Pleurocystidia very rare, similar to cheilocystidia. Stipitipellis made up of long, narrow hyphae, with frequent cylindrical cells, isolate or in clusters, caulocystidia not seen. Pileipellis made up of a cutis of interwoven hyphae, with some scattered, rising but not emerging elements. Dermatocystidia and thromboplerous hyphae absent. Clamp connections absent.

COMMENTS – The type collection is composed of an almost entire pileus and the stipe apex, all in good condition. The basidiospore size recorded in the original diagnosis is wrong, according to a personal communication by J. Raithelhuber, who states that the correct size is "10–11 × 5–7.5 µm," which was confirmed by our study of the original material.

Melanolecua lapataiae var. *ochroleuca* showed us the same micromorphology as that of *M. lapataiae* var. *lapataiae*, except for the different pileipellis structure. We think that it is correct to regard these as two taxa separated by differences in the pileipellis structure and colors of the pileus and stipe base. The peculiar cystidial morphology of these varieties prevents placement in any of the currently named subgenera and suggests erection of a new subgenus; however, because it was not possible to obtain comparable molecular sequences from the two specimens due to age, we prefer to wait for collection of fresh material before suggesting taxonomic changes.

Acknowledgments

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Anderberg, curator at S; Tuomo Niemelä, curator at H; Reinhard Berndt, curator at Z+ZT for providing us with the material requested. Our most sincere thanks are due to Pierre Arthur Moreau and Fernando Esteve Raventós for their pre-submission reviews. Edmondo Grilli is thanked for improvements in the English text.

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Coprinopsis neophlyctidospora sp. nov., a new ammonia fungus from boreal forests in Canada

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Abstract *Coprinopsis neophlyctidospora* sp. nov. (*Basidiomycota, Agaricales*), collected in urea treated soil of boreal forests from Canada is described and illustrated. Its micromorphological features, phylogenetic analysis, and mating test delineate this taxon as a new species. In addition, its ecological characters also indicate it is a new ammonia fungus.

Key words biogeography, cryptic species, ITS, species-complex, taxonomy

Introduction

The ammonia fungi are defined as a chemoecological group of fungi whose growth can be stimulated by the addition of urea or aqueous ammonia or any other related nitrogenous materials to forest soils (Sagara 1975). The diversity and ecology of ammonia fungi have been studied in diverse geographical areas such as in Japan, Taiwan, New Zealand, Western Australia, UK and western US (Suzuki et al. 2003). However, ammonia fungi in the boreal region of North America had not previously been examined. We, therefore, conducted the first survey of ammonia fungi in boreal forests of Canada. In this study, we focused on an ammonia fungus in the *Coprinopsis phlyctidospora* species-complex. *Coprinopsis phlyctidospora* had been believed to be a cosmopolitan species (Fukiharu & Horigome 1996, Suzuki et al. 2003), though we have revealed that this group represents of cryptic species (Suzuki et al. 2002) in different areas of the world. In this study, we reveal morphological characters and phylogenetic relationships based on rDNA nucleotide sequences of a new

saprobic *Coprinopsis* ammonia fungus collected from the urea treated soil of boreal forests in Canada.

Materials & methods

Isolation and culture

A mixture litter (A_0) and soil from the upper A horizon from a lodge pole pine (*Pinus contorta* var. *latifolia*) forest and from an aspen (*Populus tremuloides*) forest both near Edmonton, Canada was collected in May 2001. To stimulate fruiting of ammonia fungi, a large amount of aqueous urea (granular fertilizer; 46% nitrogen, 5–20 mg urea/g dry soil) was applied in plant pots containing the soil/litter mixture from two different forests separately and incubated at 23–25°C under a 12 hrs light/12 hrs dark regime for 30–72 days. Sterile water was applied at 1–3 day intervals to avoid desiccation of the surface of the mixture. Basidiomata were collected from plant pots and cultured in the laboratory. All cultures were grown at 25°C under a 12 hrs light/12 hrs dark regime on MY agar medium [(malt extract 10 g/L (Difco, USA), yeast extract 2g/L (Difco, USA) and agar 15g/L (Nakalai, Japan)].

Morphology

All descriptions of macro- and microscopic features were obtained from cultivated basidiomata. Different developmental stages of basidiomata were photographed and collected to observe the changes in pileus colors and veil features. Anatomical observations and measurements were made on material mounted in 25% aqueous ammonia. Microscopic terminology of basidiospores and cystidia followed that of Vellinga (1988). Color terms and notations used in this description are based on Kornerup & Wanscher (1978). Herbarium abbreviations are according to Holmgren & Holmgren (1998). Basidiospore statistics: x_m, the arithmetic mean of spore length by spore width (± standard deviation) ; n, number of spores measured; Q, the quotient of spore length and spore width; Q_m, the mean of Q-values (± standard deviation). For scanning electron microscope (SEM) observation of basidiospores samples from spore prints were rehydrated in 25% aqueous ammonia and fixed in 2.5% osmic acid, coated with platinum-palladium in an ion sputter-coater (Hitachi E-1030; Hitachi Tokyo, Japan), and observed under a SEM (Hitachi S-800) operating at 15.0 kV.

Phylogeny

Fungal strains were grown in a MY liquid medium. Mycelia were harvested, squeezed with a paper towel, frozen and lyophilized. The dried mycelia were then ground with a spatula and suspended in a TES buffer [50 mM Tris-HCl (pH 7.5), 20mM EDTA, 1% SDS] and soluble fractions were recovered by centrifugation. The DNA was purified by a TE buffer [10 mM Tris-HCl (pH 8.0), 1mM EDTA] saturated phenol/chloroform/isoamyl alcohol (Nippon gene) extraction followed by an iso-propyl alcohol precipitation. After desiccation of the DNA pellet, the DNA was dissolved in 30 µl TE buffer. For some samples, the genomic DNA was further purified using a NucleoSpin Extract II (Macherey-Nagel) following the manufacture's recommendation. Primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGTATGC-3') were used to amplify the ITS1-5.8S-ITS4 ribosomal DNA region. PCR reactions were carried out using Ex Taq (TakaraBio) according to the manufacturer's protocol. PCR

TABLE 1: *Coprinopsis* collections included in the phylogenetic analysis.

TAXON	ISOLATE NO.	VOUCHER SPECIMEN NO.	LOCALITY	DOMINATING VEGETATION	GENBANK ACCESSION NO.
Coprinopsis neophlyctidospora	CHU2009	UAMH11230*	Nojack, Alberta, CAN	*Pinus contorta* var. *latifolia*	AB564409
	CHU2021	CBM FB33899*	Chipman, Alberta, CAN	*Populus tremuloides*	AB564407
	CHU2022	CBM FB33901*	Chipman, Alberta, CAN	*P. tremuloides*	AB564406
	CHU2023	CBM FB33894*	Chipman, Alberta, CAN	*P. tremuloides*	AB564408
	CHU2024	CBM FB33896*	Chipman, Alberta, CAN	*P. tremuloides*	AB564410
	CHU2025	CBM FB38024*	Chipman, Alberta, CAN	*P. tremuloides*	AB564721
GENBANK SEQUENCES					
C. phlyctidospora △	NBRC30478**		Kyoto, Japan	*Castanopsis cuspidata*	AB071615
	CHU3010	CBM FB24544**	Chiba, Japan	*Quercus acuta, C. cuspidata*	AB071614
	CHU3017	CBM FB224548**	Chiba, Japan	*Q. acuta, C. cuspidata*	AB071616
	CHU3003	CBM FB24539*	Tokyo, Japan	*Q. acuta*	AB071609
		CBM FB21061*	Tokyo, Japan	*Q. acuta*	AB071610
C. "austrophlyctidospora" △	CHU3014	CBM FB29564*	North Island, NZ	*Nothofagus menziesii, N. fusca*	AB071793
	CHU3015	CBM FB29560*	North Island, NZ	*N. menziesii , N. fusca*	AB071794
	CHU3016	CBM FB30247*	North Island, NZ	*N. menziesii , N. fusca*	AB071795
	CHU3007**		North Island, NZ	*Pinus radiata* (Plantation)	AB071789
C. echinospora △		CBM FB21264*	Hokkaido, Japan	*Fagus crenata*	AB071798
		CBM FB21629*	Aomori, Japan	*F. crenata*	AB071799
		CBM FB21725*	Aomori, Japan	*F. crenata*	AB071800
		CBM FB21733*	Miyagi, Japan	*F. crenata*	AB071801
	CHU3019*		Kyoto, Japan	*C. cuspidata*	AB071803
OUTGROUP					
C. atramentaria	KACC49358				AF345814

* Basidiomata obtained by urea treatment in laboratory experiment; ** Basidiomata obtained by urea treatment in field experiment

△ Reference, Suzuki et al. 2002

products were purified using the NucleoSpin Extract II, and DNA fragments were directly sequenced using the BigDye Terminator ver3.1 Cycle Sequencing Kit (Applied Biosystems) according to the provided protocol. The reactions were then cleaned up using the Centri Sep (Princeton Separations) before analysis by capillary electrophoresis on a 3130x DNA Analyzer (Applied Biosystems). Sequences were assembled and edited using the ATSQ software (Genetyx). All nucleotide sequences were deposited in GenBank/EMBL/DDJB and accession numbers are provided (Table 1). The data set was aligned using Clustal X ver. 1.81 (Jeannmougin et al. 1998), and the resulting alignment was manually refined. The alignment was deposited in TreeBASE under accession number http://purl.org/phylo/treebase/phylows/study/TB2:S10848. All phylogenetic analyses were performed with Paup* 4.0b10 (Swofford 2001). Gaps were treated as missing data. The ITS data set of 730 bp was analysed with maximum parsimony. The maximum parsimony was performed with a full heuristic search with 100 random stepwise addition replicates and TBR (tree bisection-reconnection) branch swapping and equal weighting of all characters. The robustness of inferred MP tree topologies was tested by the bootstrap value (Felsenstein 1985) with 1000 replicates. *Coprinopsis atramentaria* was used as an outgroup. Sequences for *C. atramentaria, C. phlyctidospora, C.* "*austrophlyctidospora*" and *C. echinospora* were retrieved from GenBank (Table 1).

Mating tests

Mating compatibility of *C. neophlyctidospora* with *C. phlyctidospora* and *C.* "*austrophlyctidospora*" was examined. Monokaryotic testers of *C. phlyctidospora* NBRC 30478 (Suzuki et al. 2002) and *C.* "*austrophlyctidospora*" CHU 3007 (tester strains were generated by the authors of this paper, unpublished) were used in this study. Small mycelium-covered agar blocks of dikaryotic *C. neophlyctidospora* and one of the monokaryotic testers were placed 5 mm apart in the center of an MY agar plate. After about two weeks incubation at 25°C in the dark, several pieces of mycelia from the outer edge of the monokaryotic colony were examined and compatible crossings were determined by the presence of clamp connections (Tables 2, 3). Individual pairings were performed three times.

Taxonomy

Coprinopsis neophlyctidospora Raut, Fukiharu & A. Suzuki, **sp. nov.** Figs. 1–3

MycoBank 518507

Pileo primo 5–12 mm lato 3–6 mm alto, usque ad 10–30 mm lato, ovato-campanulato, demum in margine lacerato revolutoque, radiatim sulcato, candido vel eburneo, deinde cinerascenti, primo squamis albis radiatim hirsuto-fibrillosis ubique tecto, dein fere glabro; carne tenuissima, fragilissima, alba, sapore miti, odore nullo; lamellis adnexis vel liberis, comfertis, angustis (0.5–1.0 mm), albis, deinde atratis, deliquescentibus; stipete 50–80 mm longo, 1–3 mm crasso, aequali vel sursum leviter attenuato, basi leviter incrassatula, cavo, candido, fragilissimo, primo squamis albis fibrillosis squarrosis ubique tecto, dein nudo; basidiosporis in cumulo atratis, sub microscopio rufo-brunneis, 7.8–8.5 µm longis × 5.4–6.0 µm anticis × 5.4–5.8 latis, ovoideis vel ellipsoideis, verrcosis, poro germinationis centrali 1.8–2.1 µm lato, cum plagiis; basidiis 26.2–36.2 × 7.7–8.5µm, tetrasporis; pleurocystidiis 40–65 × 15–30 µm, obovatis vel utriculiformibus, hyalinis, tenui-tunicatis; cheilocystidiis 25–35 × 15–25 µm, subglobosis vel utriculiformibus, hyalinis, tenui-tunicatis; velo pilei,

FIG. 1. Macromorphology of the newly described species, *Coprinopsis neophlyctidospora*. A. Basidiomata (UAMH 11230); B. Longitudinal section of basidioma (UAMH 11230); and C E different developmental stages of basidiomata. Bars, A B 5 mm, C E 20 mm.

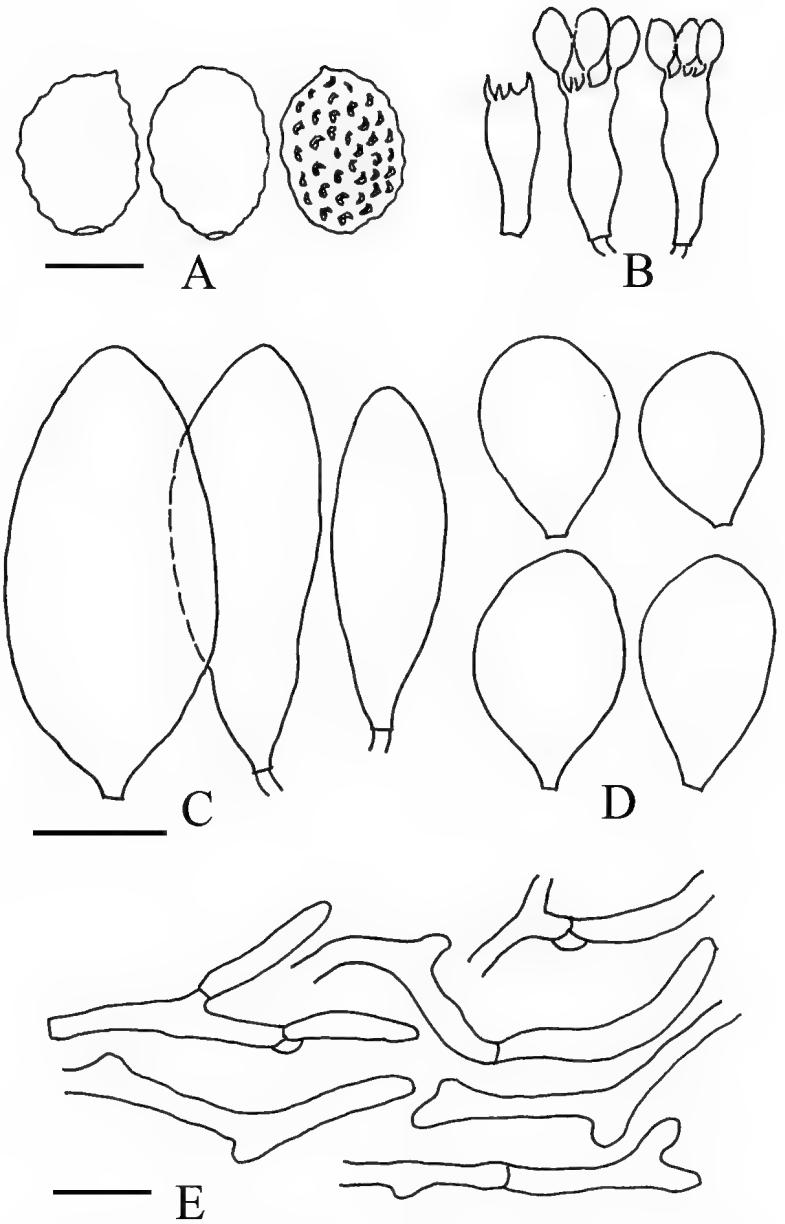

FIG. 2. Anatomical details of *Coprinopsis neophlyctidospora* (drawn from the holotype) A. Basidiospores B. Basidia C. Pleurocystidia D. Cheilocystidia D. Veil elements. Bars A 5 µm B D 20 µm, E 30 µm.

ex hyphis multi-septatis, divaricatis, tenui-tunicatis, hyalinis, 28.2 68.2 × 5.8 7.8 µm compositis; fiblis praesentibus.

TYPE: Canada, Alberta: Nojack (115°35′15"W 53°36′19"N; 828 m) on a large amount of aqueous urea (granular fertilizer; 46% nitrogen, 5 mg urea/g dry soil) applied soil/litter mixture collected from pine (*Pinus contorta* var. *latifolia*) forest, May 2001, A. Suzuki [CHU 2009] (**HOLOTYPE** UAMH 11230).

ETYMOLOGY: The Latin *neo-* refers to the "New World" origin of the species, and *-phlyctidospora* reflects its morphological resemblance to *Coprinopsis phlyctidospora*.

PILEUS 5–12 mm broad, 3–6 mm high in button stage, when young ellipsoid to ovoid, later convex to plane, 10–30 mm broad when expanded, finally uplifted with age, radially sulcate; pileipellis color at first grayish orange to pale orange (5B, 3), soon becoming grayish brown (5D-E, 3), surface when young densely covered with white, hairy fibrillose scales (Fig.1), soon breaking up in small radially arranged fibrillose veil, later almost glabrous or veil remaining only in the center. Context very thin, fragile, white; taste mild; odor indistinct. LAMELLAE adnexed to free, close (number of lamellae 40–60), with 0–1 lamellulae (between two lamellae), narrow (0.5–1.0 mm wide), edge slightly pruinose, at first white, then grayish, finally blackish, deliquescent. STIPE up to 50–80 × 1–3 mm, central, cylindrical, equal or somewhat tapering upward, sometimes the base clavate, not rooting, fistulose, fragile, surface white, at first with white fibrillose scales (Fig. 1), soon becoming smooth (Fig. 1). BASIDIOSPORES black in mass, dark reddish brown under the light microscope, 7.8–8.5 × 5.4–6.0 µm in face view (x_m = 7.9 ± 0.6 × 5.8 ± 0.5 µm, Q = 1.2–1.5, Q_m = 1.4 ± 0.1, n = 40), 5.4–5.8 µm diam in side view (x_m = 5.6 ± 0.4 µm, n = 40), ovoid to ellipsoid with warty ornamentation, a central germ pore 1.8–2.1 (1.8 ± 0.1, n = 10) µm wide and vague plage (Figs. 2, 3A). BASIDIA 26.2–36.2 × 7.7–8.5 µm, 4-spored (Fig. 3C). PLEUROCYSTIDIA 40–65 × 15–30 µm, obovoid or clavate to utriform, thin-walled, colorless (Fig. 3C). CHEILOCYSTIDIA 25–35 × 15–25 µm, subglobose or obovoid to utriform, numerous, thin-walled, colorless (Fig. 3D). ELEMENTS OF VEIL on the pileal surface composed of thin-walled, diverticulate, colorless hyphae, 28.2–68.2 × 5.8–7.8 µm (Fig. 3E). CLAMP CONNECTIONS present in vegetative mycelia and in pileal veil elements.

HABIT & HABITAT – Saprobic, growing after application of urea in the soil collected from lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) and aspen (*Populus tremuloides* Michx.) forests in Canada. Fruiting of this fungus in urea-treated soil indicates that it is an ammonia fungus (Sagara 1975).

DISTRIBUTION – Only known from the type locality in Canada.

ADDITIONAL SPECIMENS EXAMINED: CANADA. ALBERTA: Chipman, (112°45′10"W 53°39′46"N; 728 m) in a large amount of aqueous urea (granular fertilizer; 46% nitrogen, 20 mg urea/g dry soil) applied soil/litter mixture collected from an aspen (*Populus tremuloides*) forest, CBM-FB33899, CBM-FB33901, CBM-FB33894, CBM-FB33896 December 2003, T. Fukiharu, CBM-FB38024 January 2004, T. Fukiharu.

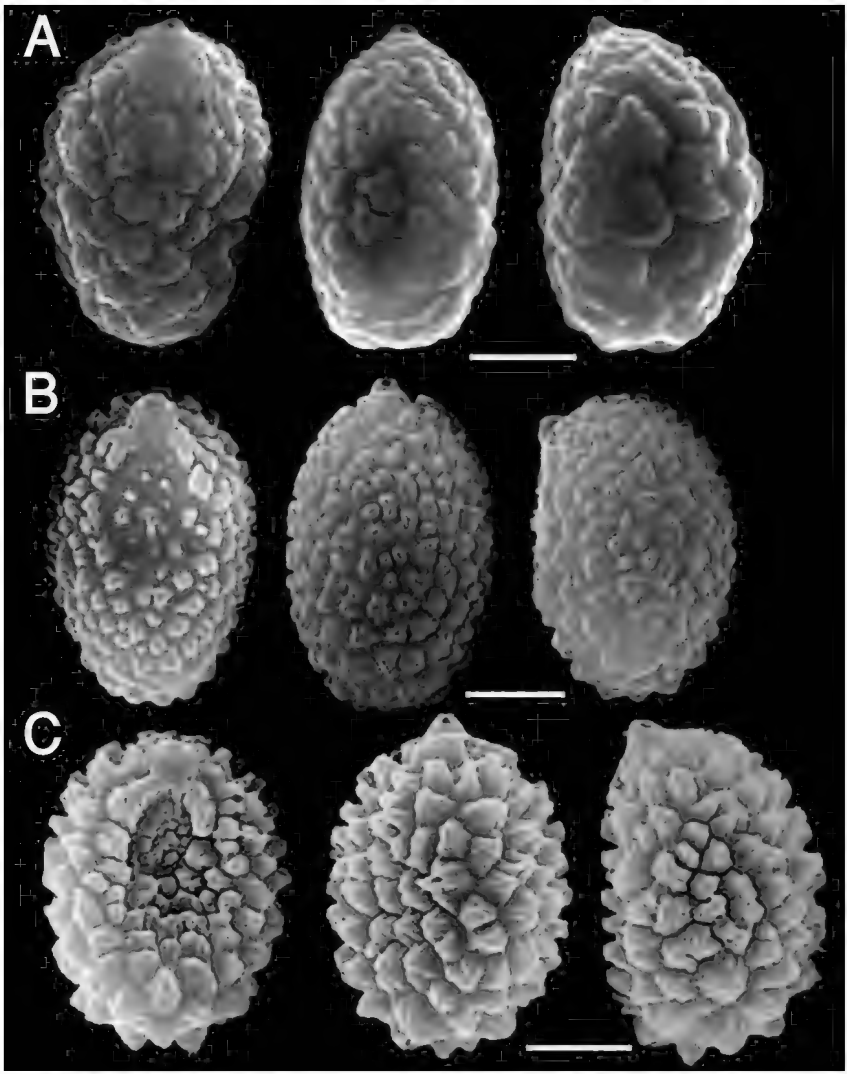

FIG. 3. Comparison of scanning electron micrographs (SEM) of basidiospores among: A. *Coprinopsis neophlyctidospora;* B. *C. "austrophlyctidospora"* (Fukiharu et al. 2011); and C. *C. phlyctidospora* (Fukiharu et al. 2011). Bars, A – C = 2.5 µm.

Results & discussion

According to conventional *Coprinus* Pers. taxonomy, *Coprinopsis neophlyctidospora* belongs to *Coprinus* subsect. *Alachuani* (Singer 1986) based on its diverticulate veil elements. In this subsection four species are reported

to have warty ornamented basidiospores, i.e., *Coprinopsis echinospora* (Buller) Redhead & al. (≡ *Coprinus echinosporus*), *Coprinopsis phlyctidospora* (Romagn.) Redhead & al. (≡ *Coprinus phlyctidosporus*), *Coprinopsis rugosobispora* (J. Geesink & Imler) Redhead & al. (≡ *Coprinus rugosobisporus*) (Orton & Watling 1979, Moser 1983, Uljé & Noordeloos 1997, Uljé 2005), and *Coprinopsis "austrophlyctidospora"* nom. prov. (proposed as a new species by Fukiharu et al. 2011). Macroscopically it is difficult to separate *C. neophlyctidospora* from these species, but some microscopic features are distinctive. *Coprinopsis echinospora* differs in its amygdaliform basidiospores and two hyphal types in the pileal veil. *Coprinopsis rugosobispora* has two-spored basidia and is also distinguished from *C. phlyctidospora* and *C. "austrophlyctidospora"*

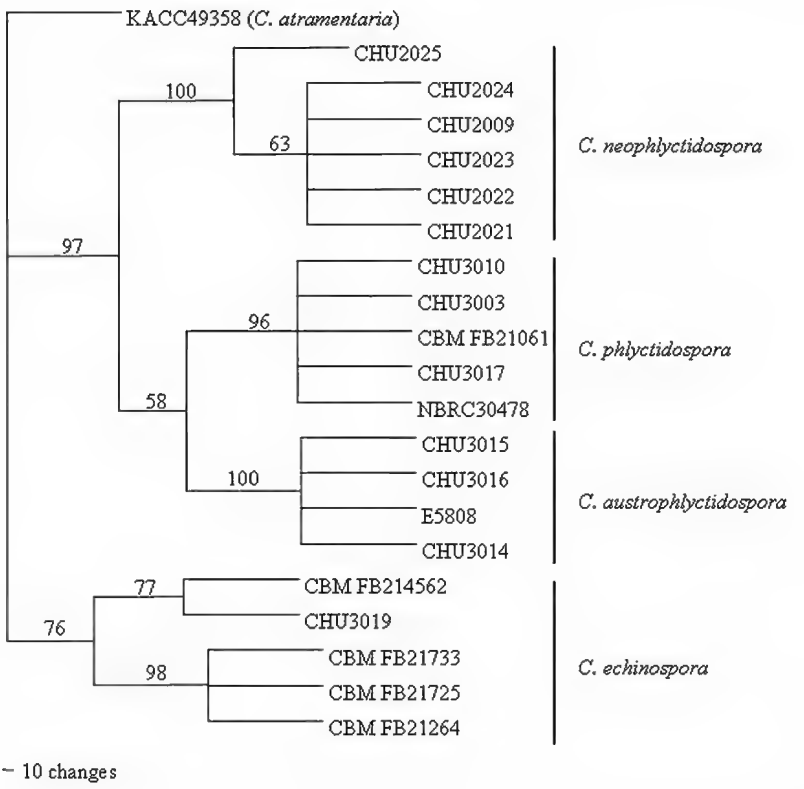

FIG. 4. The most parsimonious tree (156 steps) from a heuristic search based on the nuclear rDNA sequences in ITS region for phylogenetically related *Coprinopsis* spp. The tree was rooted to the outgroup, *C. atramentaria*. The numbers on branches indicate bootstrap support (value >50% only shown) with 1000 replications. The distance corresponding to 10 base changes per 1000 nucleotide position is indicated by the bar. Sidebar represents the inferred clades of *Coprinopsis* species.

Table 2: Dikaryon-monokaryon mating reactions between the dikaryotic isolates of *Coprinopsis neophlyctidospora* from Canada and monokaryotic testers of *C. phlyctidospora* (NBRC30478).

Dikaryotic Isolates	Monokaryotic testers of *C. phlyctidospora* from Japan							
	A_1B_1		A_2B_2		A_1B_2		A_2B_1	
	CHU003	CHU001	CHU008	CHU007	CHU010	CHU009	CHU011	CHU012
CHU2009								
CHU2021								
CHU2022								
CHU2023								

, Clamp connections did not form

Table 3: Dikaryon-monokaryon mating reactions between the dikaryotic isolates of *Coprinopsis neophlyctidospora* from Canada and monokaryotic testers of *C. "austrophlyctidospora"* (CHU3007).

Dikaryotic isolates	Monokaryotic testers of *C. "austrophlyctidospora"* from New Zealand							
	A_1B_1		A_2B_2		A_1B_2		A_2B_1	
	CHU013	CHU015	CHU021	CHU022	CHU016	CHU020	CHU018	CHU019
CHU2009								
CHU2021								
CHU2022								
CHU2023								

, Clamp connections did not form

Table 4: Diagnostic basidiospore features of *Coprinopsis neophlyctidospora* and its allies.

Basidiospore character	*C. neophlyctidospora*	*C. phlyctidospora*	*C. "austrophlyctidospora"*
Size (µm)	7.8 8.5 × 5.4 6.0	8.4 10.6 × 6.0 7.6	6.5 7.5 × 5.1 6.5
Surface (verrucose)	warts more flattened, plage not distinct	warts coarse & cone like, plage +/ distinct	warts smaller, plage always distinct

in basidiospore size and surface ornamentation. The basidiospore size of *C. neophlyctidospora* is smaller than that of *C. phlyctidospora* (8.4–10.6 × 6.0–7.6 µm; Uljé and Noordeloos 1997, Uljé 2005) while larger and more elongated than those of *C. "austrophlyctidospora"* (6.5–7.5 × 5.1–6.5 µm; Fukiharu et al. 2011). The scanning electron micrographs also revealed its distinct broadly warted basidiospores, showing warts more flattened than those in other species

(Fig. 2). Furthermore, in the phylogenetic analysis based on ITS nucleotide sequences *C. neophlyctidospora* formed a conspicuous and distinct clade among its allies. The tree topology is strongly supported by high bootstrap values (Fig. 3), indicating that *C. neophlyctidospora* is a distinct taxon. Moreover, the mating test results comparing *C. neophlyctidospora* with *C. phlyctidospora* (Table 2) and *C. "austrophlyctidospora"* (Table 3) also strongly support biological separation of these species. In conclusion, we propose *C. neophlyctidospora* as a new cryptic species within the *C. phlyctidospora* species-complex, distributed in the boreal region of Canada. Further research in unexplored regions is needed to resolve comprehensive global understanding of the distribution of these species.

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Stachybotrys thaxteri sp. nov. and the nomenclatural status of three *Stachybotrys* species

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Abstract — A new species of *Stachybotrys, S. thaxteri,* is described as producing oblong or ellipsoid conidia with a median constriction and undulating diagonal striations and colorless conidiophores up to 360 µm long. The nomenclatural status of *S. cannae,* "*S. pallida,*" and "*S. striatispora*" is clarified.

Key words anamorphic fungi, *Periconiella,* synonym

Introduction

The genus *Stachybotrys* was erected by Corda (1837: 21) with *S. atra* Corda as type species. Verona & Mazzucchetti (1968), who monographed *Stachybotrys* and *Memnoniella*, accepted 16 species of *Stachybotrys* and 3 species of *Memnoniella.* Jong & Davis (1976) published culture studies of 11 species of *Stachybotrys* and 2 species of *Memnoniella.* Pinruan et al. (2004) accepted 55 taxa in *Stachybotrys* (including seven taxa formerly placed in *Memnoniella*). At present, 96 *Stachybotrys* names have been published (Jiang & Zhang 2009, Wang et al. 2009, Index Fungorum 2010), of which ca. 44 were accepted in the 10th edition of Dictionary of Fungi (Kirk et al. 2008). Since that publication, *Stachybotrys variabilis* H.F. Wang & T.Y. Zhang (Wang et al. 2009) and *Stachybotrys pallescens* Y.L. Jiang & T.Y. Zhang (Jiang & Zhang 2009) have been added.

During study of exsiccati from the U.S. National Fungus Collections (BPI), The Farlow Herbarium (FH), Harvard University, and University of Wisconsin Herbarium, Madison (WIS), one specimen (FH 2633) collected by R. Thaxter labeled as *Stachybotrys* sp. was found to represent an undescribed species, described below as *Stachybotrys thaxteri.* During the same research, *Stachybotrys cannae* (isotype specimen BPI422138) was determined to represent

not *Stachybotrys* but *Periconiella*. The nomenclatural status of *S. cannae*, "*S. pallida*", and "*S. striatispora*" is clarified and those species are redescribed.

Materials & methods

Specimens of *Stachybotrys* on loan from BPI, FH, WIS, and several other herbaria were studied. Fungal structures including hyphae, conidiophores, conidiogenous cells, and conidia were tape-lifted from each specimen with transparent tape (2 × 3 mm) and mounted in lacto-fuchsin (0.1 g acid fuchsin, 100 mL 85% lactic acid). Morphological characters (e.g., conidiophores, conidiogenous cells, conidia) from each specimen were observed with a Nomarski differential interference contrast optical system at three different times (immediately, after one week, after one month) to determine whether rehydration had any effect on the morphology of the fungal structures. Herbarium acronyms follow Holmgren & Holmgren (1998). Articles of the International Code of Botanical Nomenclature are hereafter cited as from the "Vienna Code" (McNeill et al. 2006).

Results

Stachybotrys thaxteri D.W. Li, **anam. sp. nov.** FIGURES 1–4

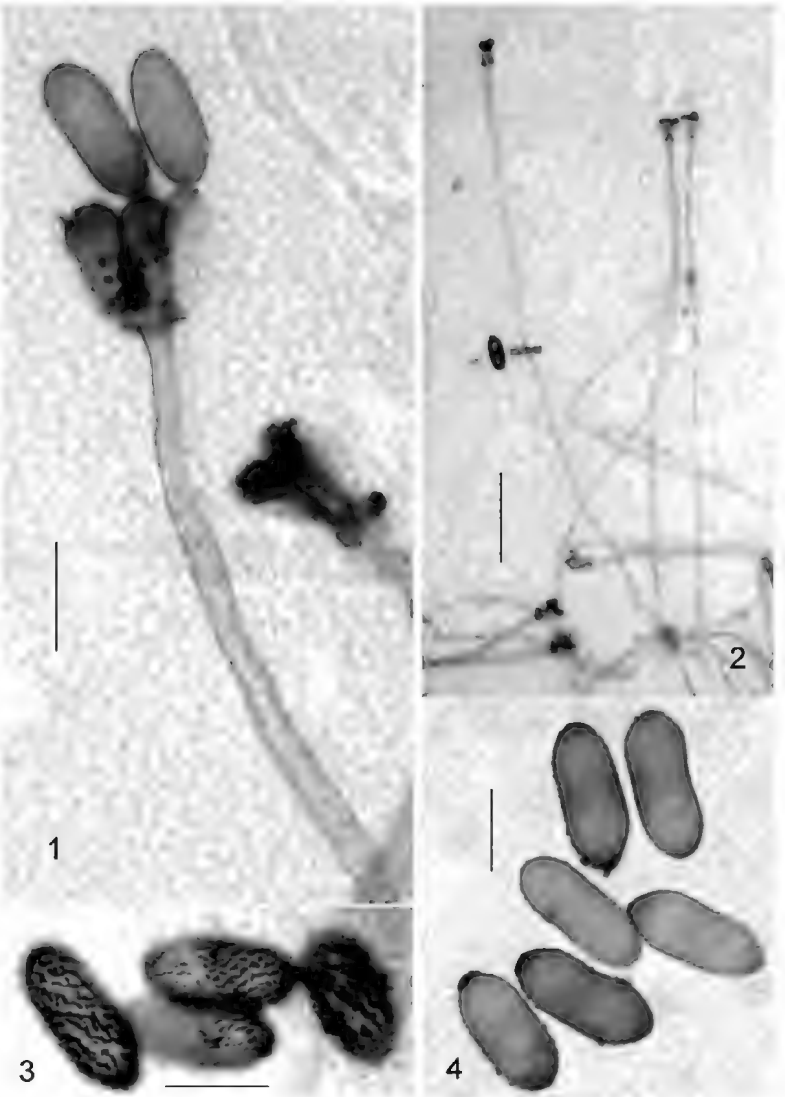
MYCOBANK MB515398

COLONIAE olivascens, effusae, velutinae. CONIDIOPHORA macronemata, erecta, simplicia, septata, solitaria vel interdum fasciculata, determinata, recta vel exigua curvata, laevia vel verrucosa, hyalina, usque ad 360 µm longa et 3.0–5.0 µm crassa. CELLULAE CONIDIOGENAE phialidicae, determinatae, discretae, laeviae, subclavatae, prope apicem fuscae olivaceae, (10.5–)11–13(–15.5) × (4.5–)5–6(–6.5) µm, collo conspicuo, praeditae, 4–6 in verticillo dispositae. CONIDIA oblonga, elliptica vel subcylindro-elliptica, primo hyalina et laevia, deinde atro-olivaceobrunnea, diagnaliter striata, (12.5–)15–17.5(–19) × (6–)6.5–7.5(–8) µm, biguttata, in massam mucosam nigram congesta. Teleomorphosis ignota.

TYPE: Trinidad: Maravel Valley, de aroid folii, per Roland Thaxter. Coll. 7-XII-1913, FH 2633 (**holotype**).

ETYMOLOGY: the specific epithet honors Roland Thaxter, the longtime curator of the Farlow Herbarium, Harvard University, who collected the holotype.

COLONIES olivaceous, effuse, and velutinous; Mycelia superficial, partially immersed. Hyphae septate, branched, light olivaceous, smooth. CONIDIOPHORES differentiated, determinate, solitary or in groups, erect, straight or flexuous, simple, 3–7-septate, smooth to verrucose, hyaline, up to 360 µm in length, 3.0–5.0 µm in width, apices inflated (FIGS. 1–2). PHIALIDES , unilocal, determinate, discrete, subclavate, smooth, hyaline, dark olivaceous at the tip, (10.5–)11–13 (–15.5) × (4.5–)5–6(–6.5) µm (mean = 12.2 ± 1.0 × 5.4 ± 0.6 µm, n = 20), with or without conspicuous collarettes, in digitate clusters of 4–6 (FIG. 1). CONIDIA unicellular, oblong, ellipsoid, occasionally constricted in the middle or slightly curved, rounded at both ends, light gray and smooth at first, gray to dark olivaceous when mature, forming delicate undulating diagonal striations with minute dots in lines on the conidial surface, (12.5–)15–17.5(–19) × (6–)6.5–7.5



FIGURES 1–4. *Stachybotrys thaxteri*. 1. Conidiophore, phialides, and conidia. 2. Long conidiophores, phialides, and conidium. 3. Conidia showing striations. 4. Biguttulate conidia. Scale bars: 1, 3, 4 — 10 μm, 2 — 40 μm.

(–8) μm (mean = 16.1 ± 1.3 × 7.1 ± 0.4 μm, n = 31), ratio of length/width 1.8–2.7 (mean = 2.3), biguttulate, especially when young, aggregated in slimy masses; the striations becoming thicker and irregular with age (FIGS. 3–4, TABLE 1).

TABLE 1. Comparative synopsis of *Stachybotrys thaxteri* and similar *Stachybotrys* species.

SPECIES	CONIDIA				CONIDIOPHORES			TYPE examined
	SIZE (μm)	SHAPE	COLOR	SURFACE	SIZE (μm)	COLOR	SURFACE	
S. chartarum	7–12 × 3.5–6	broadly ellipsoid	olivaceous	verrucose to ridged	>100 × 2.5–5, variable	brown to olivaceous	smooth, roughened or granulate	Y
S. eucylindrospora	13–16 × 3.5–5.5	cylindrical	olivaceous	striate longitudinally	<200 × 3–5	colorless to pale olivaceous	verrucose	Y
S. freycinetiae	10–15 × 4–4.5	cylindrical	black	coarsely verrucose	<320 × 5.5–8.5	colorless or pale brown	smooth or verrucose	Y
S. kampalensis	10–14 × 6–8	oblong	olivaceous	verrucose	150–250 × 8–9	subhyaline to olivaceous	smooth	Y
S. thaxteri	15–18 × 7–7.5	oblong, ellipsoid constricted at middle	olivaceous	striate diagonally	<360 × 3–5	colorless	smooth to verrucose	Y
S. theobromae	20–28 × 15–18	oval with an apiculus	black	smooth to verrucose	<200 × 4–6	colorless	smooth	Y
S. variabilis	4–20 × 3–13	subglobse to ellipsoid	pale brown to dark brown	smooth to verrucose	45–70 × 3–4	subhyaline to brown	smooth to verrucose	N
S. virgata	12–13 × 5–5.5	ellipsoid	dark brown	verrucose	100 × 4.5	brown	coarsely verrucose	N
S. xanthosomatis	10–19 × 5–6.5	ellipsoid to cylindrical	olivaceous	coarsely verrucose	120–260 × 3–4	olivaceous	verruculose	N
S. waitakere	15–19 × 8–12	ellipsoid base rounded or with a truncate papilla	black	verrucose	54–75 × 4.5–7	colorless to gray at apex	smooth	N

TELEOMORPH: unknown.

DISTRIBUTION AND HABITAT: Known only from Trinidad on aroid leaves.

ADDITIONAL TYPE SPECIMENS OF SIMILAR *STACHYBOTRYS* SPECIES EXAMINED: **Canada.** ONTARIO: Near Guelph, from peat soil, cedar bog, Nov. 1960, G.L. Barron [DAOM 87664, type of *Stachybotrys eucylindrospora*]. **Czech Republic.** PRAGUE, on plaster, A.C.J. Corda [PRM 155670, holotype of *Stachybotrys atra* Corda]. **Germany.** BERLIN. Unknown locality, on paper, date, collector unknown [Herb. Inst. Syst. Bot. Uppsala, Sweden. Herb. *E. Fries*, ex holotype of *Stilbospora chartarum* Ehrenb. (≡ *Stachbotrys chartarum*) (DAOM 51026)]. **New Zealand.** WAIKATO. Mt. Pirongia, No. 3 Track, on *Freycinetia banksii* A.Cunn., 19 Nov 1984, E.H.C. McKenzie [PDD 57639, holotype of *Stachybotrys freycinetiae*]. **Uganda.** KAMPALA: on dead wood, March 1930, C.G. Hansford (1138) [K(M) 165379, holotype of *Stachybotrys kampalensis* Hansf.]; on dead branches of *Theobroma cacao* L., May 1936, C.G. Hansford (1138), [K(M) 165384, holotype of *Stachybotrys theobromae* Hansf.].

COMMENTS—A comparison of *S. thaxteri* and similar *Stachybotrys* species is given in TABLE 1.

One striking character of *S. thaxteri* is the long (≤ 360 µm) conidiophore. Both *S. freycinetiae* McKenzie and *S. xanthosomatis* Mercado & J. Mena also have long conidiophores. The holotype of *S. freycinetiae* has conidiophores up to 320 µm long, but its conidia are cylindrical and much smaller (10–15 × 4–4.5 µm; McKenzie 1991) than those of *S. thaxteri*. *Stachybotrys xanthosomatis* produces 120–260 µm long conidiophores and conidia with extremely variable lengths (Mercado & Mena 1988) that overlap those of *S. thaxteri*, but from which it is distinguished by its narrower, ellipsoid or cylindrical conidia (10–19 × 5–6.5 µm). The conidial widths of the two species do not overlap (TABLE I). *Stachybotrys virgata* has warted conidiophores up to 100 µm long and ellipsoid conidia that are smaller (12–13 × 5–5.5 µm; Krzemieniewska & Badura 1954) than those of *S. thaxteri*. Although the type specimens of *S. xanthosomatis* and *S. virgata* were deposited in herbaria in Cuba and Poland and thus unavailable to the author, the literature descriptions of these two species are detailed enough for comparison.

Conidia of several other species are similar in size or shape to those of *S. thaxteri*. Conidia of *S. waitakere* Whitton et al. are similar in size (14.5–19 × 8–11.5 µm) but differ in ornamentation (verrucose) and shape (ellipsoid to broadly ellipsoid) and are produced on short (≤ 75 µm) conidiophores (Whitton et al. 2001). Conidia of *S. kampalensis* are morphologically similar to those of *S. thaxteri* but smaller (10–14 × 6–8 µm; Jong & Davis 1976; holotype examined). The conidia of *S. theobromae* (20–28 × 15–18 µm; Hansford 1943; holotype examined) and *S. variabilis* (4–20 × 3–13 µm; Wang et al. 2009) overlap those of *S. thaxteri* in size. However, the larger ovoid conidium with the apical apiculus of *S. theobromae* separates that species from *S. thaxteri*.

In *S. variabilis* some conidia are oblong, but smooth to verrucose (not striate) and the size and shape variability combined with the short (45–70 µm)

conidiophores easily separate this species from *S. thaxteri*. Although conidia of *S. thaxteri* and *S. eucylindrospora* are both striate, the longitudinal striations on the smaller (12.8–16 × 3.5–5.5 μm) cylindrical conidia of *S. eucylindrospora* readily distinguish that species from the larger conidia with diagonally oriented striations in *S. thaxteri* (TABLE 1) (Li 2007).

A culture of *S. thaxteri* was not isolated when the specimen was collected. Therefore DNA sequencing could not be conducted to determine its phylogenetic relationship with the species of *Stachybotrys* that have DNA sequence data in GenBank.

Nomen Excludendum

Stachybotrys cannae Bat.

Examination of this specimen showed that its conidiogenous cells were clearly sympodial and cicatrized, rather than phialidic, and that the fungus had been misdetermined. It is identical with *Periconiella portoricensis,* which is redescribed as follows:

Periconiella portoricensis (F. Stevens & Dalbey) M.B. Ellis, Mycol. Pap. 111: 26 (1967) FIGURES 5–12

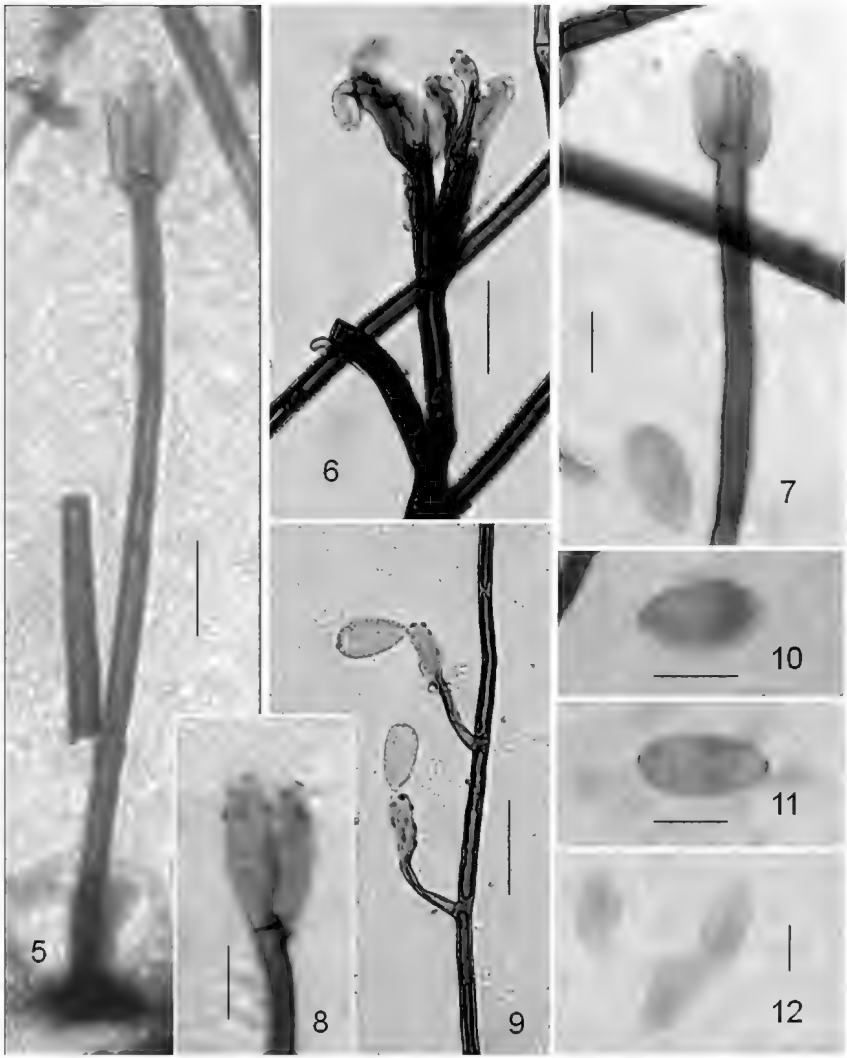
≡ *Haplographium portoricense* F. Stevens & Dalbey, Mycologia 11(1): 6 (1919).

≡ *Cephalotrichum portoricense* (F. Stevens & Dalbey) Toro, Sci. Survey Porto Rico Virgin Islands, New York Acad. Sci. 8: 224 (1932).

Stachybotrys cannae Bat., in Batista & Vital, Anais Soc. Biol. Pernambuco 15(2): 394 (1957).

COLONIES brown, velutinous, effuse. Mycelium superficial, partially immersed. Hyphae septate, branched, light brown, smooth. CONIDIOPHORES differentiated, solitary or in groups, septate, erect, straight or flexuous, smooth, brown to dark brown, thick-walled, occasionally branched, up to 430 μm in length, 4–6.5 μm in width, with a defined foot cell (FIGS. 5–6). CONIDIOGENOUS CELLS clavate or cylindrical, polyblastic, sympodial, cicatrized, smooth, brown, thick-walled in basal area, aggregated in groups of 2 – 5 on the apex of the conidiophores or solitary; solitary ones longer than these in groups; aggregated conidiogenous cells (14–)17–23(–25) × (4.5–)5–7(–8.5) μm (mean = 20.6 ± 3 × 6.1 ± 0.9 μm, n = 20) μm, fertile part as wide as the basal part (FIGS. 5–8); solitary conidiogenous cells terminal or intercalary, (19–)23–34(–43.0) × (5–)5.5–7(–7.5) μm (mean = 29.2 ± 5.5 × 6.3 ± 0.8 μm, n = 15) μm, fertile part often wider than the basal part (Figure 9). CONIDIA unicellular, acropleurogenous, single or in short chains, obovoid, ellipsoid or subcylindrical, brown, smooth or rarely verruculose, (15–)16–20(–22) × (6–)7.5–10(–13) μm (mean = 18.2 ± 1.9 × 9.2 ± 1.5 μm, n = 31) μm, ratio of length/width 1.5–3.2 (mean = 2.0), cicatrized on basal end, occasionally on both ends (FIGS. 10–12). Teleomorph unknown.

TELEOMORPH: unknown.



FIGURES 5–12. *Periconiella portoricensis*. 5–6, 8. Conidiophores, and conidiogenous cells in groups. 7. Conidiophore, conidiogenous cells in a group, and conidium. 9. Solitary conidiogenous cells and conidia. 10–12. Conidia. Scale bars: 7, 8, 10–12 = 10 µm, 5, 6, 9 = 20 µm.

DISTRIBUTION AND HABITAT: Known from Ecuador, Guyana and Puerto Rico on *Canna coccinea* Mill. and *Canna* sp.

SPECIMENS EXAMINED: **Puerto Rico.** AIBONITE: on *Canna coccinea*, 16 July 1915, J. A. Stevenson 8447 [ILL 15999 (= Herbarium of John Stevenson, paratype of *Haplographium portoricense*]; EL GIANTE: on *Canna* sp., 16 July 1915, J.A. Stevenson 8495 [ILL 16000 (=

Herbarium of John Stevenson), holotype of *Haplographium portoricense*]; GUAYNABO: on leaves of *Canna* sp., September 1917, J.A. Stevenson 6608 [BPI 422138 (Herbarium of John Stevenson), isotype of *Stachybotrys cannae*].

COMMENTS—The genus *Periconiella* was erected by Saccardo (Saccardo & Berlese 1885: 727). It is characterized by differentiated conidiophores having a branched head with polyblastic, sympodial, conspicuously cicatrized conidiogenous cells (Ellis 1967). Batista & Vital (1957) proposed *Stachybotrys cannae* as a new species. However, its possession of polyblastic, sympodial conidiogenous cells, rather than phialides, indicates that it should be classified in *Periconiella* rather than in *Stachybotrys*. Unlike *Stachybotrys*, which is defined as having — without exception — unicellular conidia, the conidia of *Periconiella* species are transversely non- to multi-septate (Ellis 1971). Examination of the *Stachybotrys cannae* and *Periconiella portoricensis* type materials led the author to conclude that these two species are conspecific due to their shared conidiogenesis, conidial shape and size, as well as habitat and distribution. Conidia of *S. cannae* were 16–20 × 7.5–10 µm, while those of *P. portoricensis* are 17–20 × 7–10 µm. Since *H. portoricense* (published in 1919) has priority over *S. cannae* (published in 1957), *Periconiella portoricensis* is the correct name of *S. cannae* (Vienna Code Art. 11.4).

Periconiella portoricensis was first described as *Haplographium portoricense*. Toro (1932) transferred this species from *Haplographium* to *Cephalotrichum*. However, this transfer was incorrect, because the species of *Cephalotrichum* have annellidic (percurrently proliferating) conidiogenesis and synnematous conidiophores (Kirk et al. 2008). Ellis (1967) proposed a new combination, transferring *H. portoricense* to *Periconiella*. Because the description and illustrations by Stevens & Dalbey (1919) and Batista & Vital (1957) were not detailed enough to show clearly diagnostic characters such as conidial orientation on conidiogenous loci, photographic illustrations and a detailed description are provided here.

"*Acrodesmis portoricensis* (F. Stevens & Dalbey) M.B. Ellis ined." is listed as an obligate synonym of *Periconiella portoricensis* in Mycobank. Since "ined." refers to an unpublished name, "*Acrodesmis portoricensis*" is nom. inval.

Nomina Invalida

"*Stachybotrys pallida*" Orpurt, Studies on the soil microfungi of Wisconsin prairies. Ph.D. dissertation, University of Wisconsin, pages 95–96 (1954), nom. inval. (Vienna Code, Art. 30.5 and 36.1).

The valid name for this taxon is:

Stachybotrys elegans (Pidopl.) W. Gams, Compendium of Soil Fungi (London): 746 (1980).

≡ *Hyalobotrys elegans* Pidopl., Fungus flora of coarse fodder: 186 (1948).

Hyalostachybotrys bisbyi Sriniv., J. Indian Bot. Soc. 37: 340 (1958).

≡ *Stachybotrys bisbyi* (Sriniv.) G.L. Barron, Mycologia 56: 315 (1964).

Hyalostachybotrys sacchari Sriniv., J. Indian bot. Soc. 37: 341 (1958).

≡ *Stachybotrys sacchari* (Sriniv.) G.L. Barron, Mycologia 56: 315 (1964).

Stachybotrys aurantia G.L. Barron, Can. J. Bot. 40: 258 (1962).

Specimens examined: **Canada**. British Columbia: Vancouver International Airport, Straw from interior of cushings shipped from Thailand and seized at the airport, 13 January 1998, G. White, [DAOM 225565 (labeled as *S. bisbyi*)]. Ontario: from soil, J.A. Traquair, [DAOM 222969]. Ottawa, isolated from serially washed roots of yellow birch, November 1961, E.A. Peterson, [DAOM 87338 (labeled as *S. bisbyi*)]. Ottawa, CEF, from soil, 18 August 1998, D. Overy, [DAOM 226830]. **Egypt**. from soil, 16 December 1965, A.H. Moubasher, [IMI 116426 (labeled as *S. sacchari*)]. **India**. Gwalior, from soil, 15 September 1976, R.K.S. Chauhan, [IMI 206921]. **Kenya**. Nairobi, from *Isoptera* (*Insecta*), 7 November 1985, M.O. Kotengo [IMI 299091]. **Pakistan**. from *Linum usitatissimum* L., 1965, M. Kamal, [IMI 114745 (labeled as *S. sacchari*)]. **South Africa**. from *Saccharum officinarum* L., 19 January 1962, J.R. Anderson, [IMI 91211 (labeled as *S. sacchari*)]. **USA**. Colorado: Fort Collins, isolated from rhizosphere of *Agropyron repens* (L.) P. Beauv., 1969, L.W. Durrell, [DAOM 129721 (labeled as *S. bisbyi*)]. New York: Ithaca, isolated from soil, May 1962, W.J. Jooste, [DAOM 89199 (2 packets) (labeled as *S. bisbyi*)]. Wisconsin: Columbia County, Morrisonville, isolated from soil of Mesic Prairie, on corn meal potato dextrose, 6 August 1952, P.A. Orpurt, [(WIS 55), "holotype" of "*Stachybotrys pallida*"].

Comments— Examination of the type specimen of "*S. pallida*" (WIS 55) revealed only mycelia, with both conidia and phialides lacking. The nomenclatural status of "*S. pallida*" is therefore based on the English description and accompanying illustrations in Orpurt's dissertation. "*Stachybotrys pallida*" (= *S. elegans*) was frequently isolated from the soils of the prairie in Wisconsin (Orpurt & Curtis 1957).

"*Stachybotrys striatispora*" Orpurt, Studies on the soil microfungi of Wisconsin prairies. Ph.D. dissertation, University of Wisconsin, pages 93–94 (1954), nom. inval. (Vienna Code, Art. 30.5 and 36.1).

The valid name for this taxon is:

Stachybotrys eucylindrospora D.W. Li, Mycologia 99(2): 333 (2007).

Specimens examined: **Canada**. Alberta: Waterton National Park, Aspen parkland picnic area, on *Populus* log, 4 August, 1980, G.P. White-696, [DAOM 176800a (2 packets)]. British Columbia: South Burnaby, *Dahlia* stems, 9 August 1957, S.J. Hughes, [DAOM 56386d (2 packets)]. Ontario: near Guelph. From peat soil, cedar bog, Nov. 1960, G.L. Barron, [DAOM 87664, holotype of *Stachybotrys eucylindrospora*]; Ottawa, on stems of "*Ligustrum officinale* Lilach" (invalid pre-Linnaean name), 26 May 1983, W.I. Illman, [DAOM 186941]; St. Lawrence Islands National Park, MacDonald Is. *Carya ovata* husks, 23 July 1979, S.J. Hughes, [DAOM 172376g]. Quebec: MacDonald College, from soil, 1 June 1955, O.A. Olsen, [DAOM 70309 (as *S. atra*)]. **United Kingdom**: England—Yorkshire: Gundale, from *Angelica* sp., 18 October 1959, W.G. Bramley, [IMI 79062 (labeled as *S. atra*)]; Pickering, from *Heracleum* sp., 3 July 1964, W.G. Bramley, [IMI 107222]. **USA**. Wisconsin: Racine County, Mesic Prairie soil (isolated on

corn meal potato dextrose), 23 August 1952, P.A. Orpurt, [(WIS 56 319-13), "holotype" of "*Stachybotrys striatispora*"].

Additional specimens examined: **Israel**. Beeshaba, soil, September 1953, *S. Borat*, [IMI 76515, (labeled as *Stachybotrys atra* var. *cylindrospora* (C.N. Jensen) Rayss & Borut 1958 (*S. chartarum*), the specimen on which the new combination was based)]. **USA**. New York, North Cohocton, arable soil, August 1911, C.N. Jensen, [CUP 5925, holotype of *Stachybotrys cylindrospora* (*S. chartarum*)].

Comments—According to Orpurt (1954) "*S. pallida*" was a common species found throughout the prairie in Wisconsin, while "*S. striatispora*" (= *S. eucylindrospora*) was isolated from only two locations. The former species was occasionally isolated from indoor environments.

Although "*S. striatispora*" was proposed much earlier than *S. eucylindrospora*, *S. eucylindrospora* is the valid name for this species due to the nomenclaturally invalid status of "*S. striatispora*". Orpurt (1954) examined a slide prepared by Dr. Richard Korf from the holotype of *Stachybotrys cylindrospora* C.N. Jensen as suggested by Dr. G.R. Bisby. He concluded that *S. cylindrospora* is a synonym of *S. atra* (= *S. chartarum)*. This conclusion is in agreement with the same conclusion drawn by three other authors (Bisby 1943, Gilman 1945, Li 2007).

Discussion

Examination of the specimens from several major herbaria including 31 types has clarified the circumscriptions of the species presented in this paper. In the author's opinion, delineation of several *Stachybotrys* species, such as *S. lunzinensis* Szilvinyi, remains doubtful due to the sketchy descriptions and/or unavailable illustrations and type specimens. Further studies on these doubtful species are needed. Without examining type materials of the species in question or sequencing their DNA (if a culture were available), a nomenclature change would be preliminary and thus add to the confusion.

Stachybotrys cannae may not be the last species transferred to a different genus. One challenge is the availability and accessibility of type materials collected in the 19th and early 20th centuries. A number of types collected at much later times are missing or were destroyed by natural disaster, such as the Kobe earthquake in Japan in 1995 (Matsushima pers. comm. 2008).

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USDA (NRRL), Auburn University (AUA), University of Alberta (UAMH), Herbarium of Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS) for specimen loans and to BPI and FH for providing the access to the mycological libraries in their institutions for this study. The author is very grateful to Dr. Lorelei L. Norvell for her editorial review, Dr. Shaun Pennycook for his nomenclature review, Dr. Weidong Wu for scanning the descriptions of *Stachybotrys* species from Orpurt's dissertation, and support from Jiangsu Natural Science Foundation (BK2008243).

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Two new species of *Phialophora* from soil

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ABSTRACT Two new species, *Phialophora microspora* and *P. nielamuensis*, both from soil in China are described and illustrated. The type specimens (dried cultures) and living cultures are deposited in the Herbarium of Shandong Agricultural University Plant Pathology (HSAUP). Isotypes are kept in the Herbarium of Institute of Microbiology, Academia Sinica (HMAS).

KEY WORDS taxonomy, soil fungi, dematiaceous hyphomycetes

Introduction

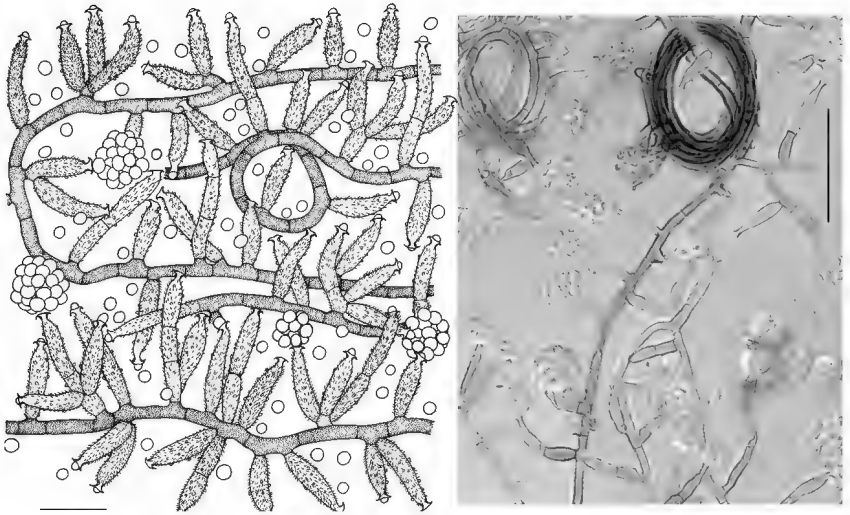
Since *Phialophora* Medlar was erected in 1915, 86 epithets have been proposed in the genus (Anonymous 2010). This genus is characterized by possession of pigmented or hyaline colonies, weakly pigmented or hyaline, thin-walled, branched conidiophores or simple phialides (which are discrete, typically flask-shaped, with a distinct collarette), and one-celled, hyaline or slightly pigmented conidia. In the course of a survey of soil dematiaceous hyphomycetes in China, several unusual species of *Phialophora* were collected. Two of them are described as new species, *P. microspora* and *P. nielamuensis*.

Taxonomy

Phialophora microspora Y.M. Wu & T.Y. Zhang, **sp. nov.** FIG. 1

MYCOBANK MB 518896

Coloniae in PDA velutinae, leviter radiatis sulcais, griseo-olivaceae. Hyphae ramosae, septatae, olivaceae, laeves vel modice scabrae, 2 3 µm crassae. Conidiophora micronemata vel semi-macronemata, mononemata, erecta, ex hyphis oriunda, simplicia, dilute brunnea, laevia, verrucosa, 30 80 × 4 7 µm. Cellulae conidiogenae monophialidicae, terminales vel laterales, ex hyphis statim et singulatim oriundae, discretae, subcylindricae, gradatim attenuatae versus apicem, subcollari modice angustatae, dilute brunneae, verrucosae, 20 30 × 4 7 µm, nonnumquam percurrenter proliferantiae; colla conspicua, infundibuliformia



F IG. 1. Conidia, conidiophores, and conidiogenous cells of *Phialophora microspora*. Left: drawings. Right: photomicrographs (Bars = 25 µm).

vel ureceolata, paulo-obscura. Conidia unicellularia, globosa vel subglobosa, pallide olivacea, hyalina, laevia, 2–3 × 1.5–2.5 µm, coacervata in mucosa capitula.

HOLOTYPE: China. Tibet, Nielamu, from a mountain soil, altitude 2600 m, 14 Sept. 2007, Y.M. Wu, HSAUPII$_{07}$1381, **holotype**; HMAS 196259, **isotype**.

ETYMOLOGY: The epithet refers to the small conidia of this species.

Colonies on PDA at 25°C for 28 days 3–5 cm diam., velvety, with faint radial furrows, gray-olivaceous. Mycelium mostly superficial, partly immersed. Hyphae branched, septate, smooth or sometime roughened, olivaceous, 2–3 µm wide. Conidiophores semi-macronematous, mononematous, erect, arising from the hyphae, simple or occasionally branched, light brown, verrucose, 30–80 × 4–7µm. Conidiogenous cells monophialidic, terminal and integrated or discrete borne directly on the hyphae, subcylindrical, gradually tapering towards the apex and more or less constricted below the collarette, light brown, verrucose, 20–30 × 4–7 µm, sometimes proliferating percurrently; collarettes conspicuous, funnel to vase-shaped, slightly darker. Conidia one-celled, globose or subglobose, hyaline, smooth, 2–3 × 1.5–2.5 µm, aggregated in slimy heads.

Phialophora microspora resembles *P. richardsiae* (Nannf.) Conant (Conant 1937) and *P. benthica* K.R. Millar (Millar 1990) in conidial colour and shape, but the conidiogenous cells of these species are smooth, and the conidia of *P. microspora* are smaller than those of either *P. benthica* (2.5–4 × 2–2.5 µm) or *P. richardsiae* (2.7–3.3 µm) diam.

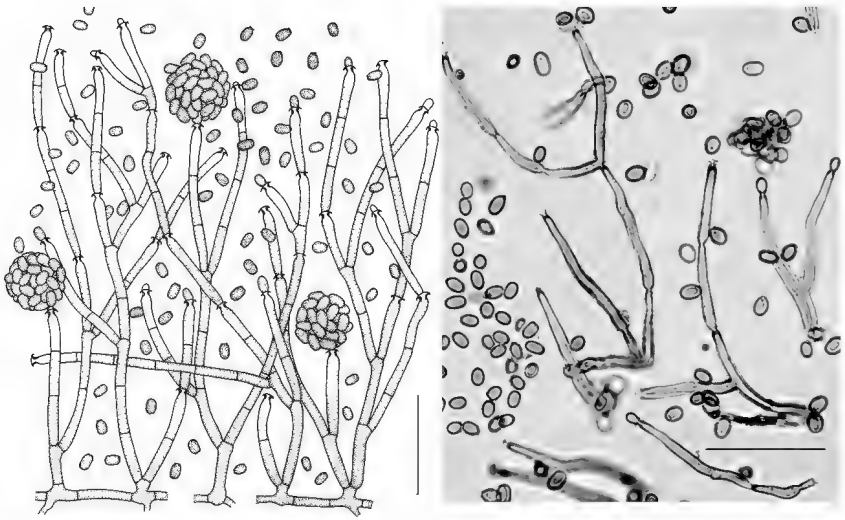


FIG. 2. Conidia, conidiophores, and conidiogenous cells of *Phialophora nielamuensis*. Left: drawings. Right: photomicrographs (Bars = 25 µm).

Phialophora nielamuensis Y.M. Wu & T.Y. Zhang, **sp. nov.** FIG. 2

MYCOBANK MB 518895

Coloniae in PDA velutinae, leviter radiatis sulcatis, griseo-olivacea. Hyphae ramosae, septatae, olivaceae vel dilute bruneae, laeves, 1.5–3 µm crassae. Conidiophora micronemata vel semi-macronemata, mononemata, erecta, ex hyphis oriunda, simplicia, dilute brunnea, laevia, 20–110 × 1.5–3 µm. Cellulae conidiogenae monophialidicae, terminales vel laterales, ex hyphis statim et singulatim oriundae, discretae, subcylindricae, gradatim attenuatae versus apicem, subcollari modice angustatae, dilute brunneae vel subhyalinae, laevia, 15–60 × 1.5–3 µm, nonnumquam percurrenter proliferantiae; colla conspicua, infundibuliformia vel ureceolata, paulo-obscura. Conidia unicellularia, pyriformia, ellipsoidea vel subglobosa, hilo basilari plano, pallide olivacea, laevia, 3.5–5 × 2–2.5 µm, coacervata in mucosa capitula.

HOLOTYPE: China. Tibet, Nielamu, from a grassland soil, altitude 2300 m, 14 Sept. 2007, Y.M. Wu, HSAUPII $_{07}$1354, **holotype**; HMAS 196258, **isotype**.

ETYMOLOGY: in reference to the type locality.

Colonies on PDA at 25°C for 28 days 3–5 cm diam., velvety, with faint radial furrows, gray-olivaceous. Mycelium mostly superficial, partly immersed. Hyphae branched, septate, smooth, olivaceous to light brown, 1.5–3 µm wide. Conidiophores micronematous to semi-macronematous, mononematous, erect, arising from the hyphae, sometimes branched light brown, smooth, 20–110 × 1.5–3 µm. Conidiogenous cells monophialidic, integrated and terminal, or discrete, subcylindrical, gradually tapering towards the apex and more or less constricted below the collarette, light brown to subhyaline, smooth, 15–60

× 1.5–3 µm, sometimes proliferating percurrently; collarettes conspicuous, funnel to vase-shaped, slightly darker. Conidia one-celled, pyriform, ellipsoidal or subglobose, with a flat basal hilum, pale olivaceous, smooth, 3.5–5 × 2–2.5 µm, aggregated in slimy heads.

This fungus resembles *Phialophora japonica* Iwatsu & Udagawa (Iwatsu & Udagawa 1985) and *P. taiwanensis* Matsush. (Matsushima 1983) in conidial morphology. However the former species has smaller conidia (1.5–3.5 × 1.2–2 µm), and the latter has larger (4.5–7 × 2–2.8 µm), darker conidia.

Acknowledgments

The authors are grateful for pre-submission comments and suggestions provided by Dr. Eric McKenzie, Prof. Y.L. Guo, and Dr. Shaun Pennycook. This project was supported by the National Science Foundation of China (no. 30670014 & 30499340).

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Phlebiopsis mussooriensis (*Agaricomycetes*), a new corticioid species from India

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ABSTRACT – A new corticioid species, *Phlebiopsis mussooriensis*, is described from Mussoorie in Uttarakhand.

KEY WORDS – Company Garden, *Cedrus deodara*

While conducting the fungal foray in the Company Garden in Mussoorie of Uttarakhand, India, Dhingra and Navneet made a collection from a decaying log of *Cedrus deodara*. On the basis of macroscopic and microscopic characters it was compared with species of *Phlebiopsis* and *Phanerochaete* (Eriksson et al. 1978 1981, Burdsall 1985, Dhingra 1987, Parmasto et al. 2009). As it could not be assigned to any of the known taxa in these genera, it is here described as a new species. With the addition of the new species to the four earlier reported species, the total number from India has become five, a key to which is also provided.

Phlebiopsis mussooriensis Priyanka, Dhingra & N. Kaur, **sp. nov.** FIGS 1 5

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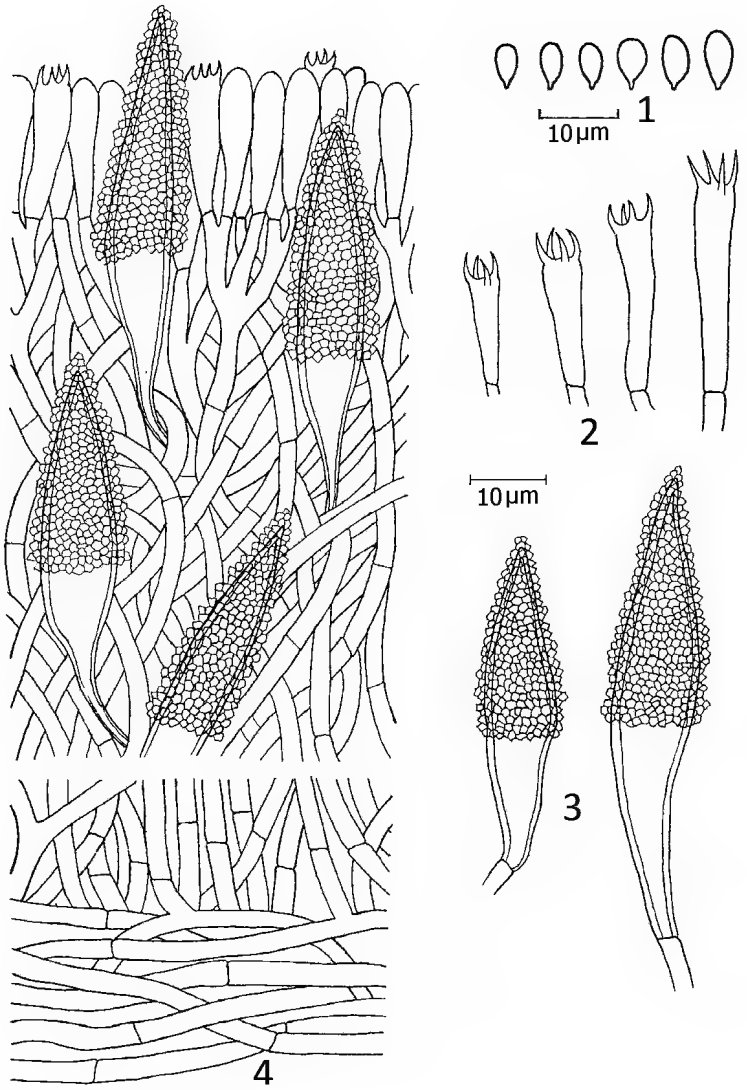
Basidiocarpum resupinatum, adnatum, effusum, ad 560 µm crassum; hymenium tuberculatum; systema hyphale monomiticum; hyphae ad 3.6 µm latae, fibulae destitutae; cystidia 40.0 62.0 × 7.2 10.9 µm, subfusiformia vel fusiformia, encrustata; basidia 13.0 26.7 × 3.3 5.0 µm, clavata, 4-sterigmata; basidiosporae 5.0 7.1 × 2.5 3.8 µm, ellipsoidae, tenuitunicatae, inamyloidae, acyanophilae.

TYPE: India, Uttarakhand: Mussoorie, Company Garden, on a decaying log of *Cedrus deodara* (Roxb.) G. Don (*Pinaceae*), Navneet 3405 (PUN, **holotype**), September 26, 2004.

ETYMOLOGY: The epithet refers to 'queen of hills,' Mussoorie, in Uttarakhand where the holotype was collected.



Figs 1–4. *Phlebiopsis mussooriensis*: microscopic structures
1. basidiospores; 2. basidia; 3. cystidia; 4. vertical section through basidiocarp.

FIG. 5. *Phlebiopsis mussooriensis*: basidiocarp showing hymenial surface.

Basidiocarp resupinate, adnate, effused, up to 560 µm thick in section; hymenial surface smooth to somewhat tuberculate, grayish yellow, not changing to purplish on putting a drop of 3% KOH solution, cracks appearing on drying; margins thinning out to abrupt. Hyphal system monomitic; generative hyphae up to 3.6 µm wide, branched, septate, without clamps; basal zone up to 290 µm thick, composed of compactly packed hyphae running parallel to the substratum; subhymenium of irregularly branched, compactly packed vertical hyphae; hymenium thickening with evenly distributed cystidia. Cystidia 40.0–62.0 × 7.2–10.9 µm, numerous, subfusiform to fusiform, thin- to somewhat thick-walled, especially at the base, naked when young to heavily encrusted in the upper ⅔ portion when mature, immersed or projecting up to 45 µm from the hymenium. Basidia 13.0–26.7 × 3.3–5.0 µm, narrowly clavate, apically widened, thin-walled, 4-sterigmate, without basal clamp; sterigmata up to 5.0 µm long. Basidiospores 5.0–7.1 × 2.5–3.8 µm, ellipsoid, smooth, thin-walled, inamyloid, acyanophilous.

REMARKS—*Phlebiopsis mussooriensis* resembles members of the genus *Phanerochaete* in producing a thickened hymenium with evenly distributed encrusted cystidia, but the newly described species differs from the same by a distinctly firm subiculum composed of narrow, thin-walled hyphae (up to 3.5 µm wide). On the other hand, it is quite similar to *Phlebiopsis himalayensis*

Dhingra in producing a smooth to somewhat tuberculate basidiocarp and thick-walled encrusted cystidia, but the basidiospores of the latter species are considerably shorter (3.5–4.75 × 2.5–3.0 µm) than in the new species. In addition, basidiocarp of *P. himalayensis* turns purplish on putting a drop of 3% KOH solution, a reaction that is not observed for *P. mussooriensis*. The new combination of features supports an independent species.

Consolidated key to the Indian species of *Phlebiopsis*:

1. Cystidia not massive (generally up to 10 µm wide) 2
1. Cystidia massive (more than 10 µm wide) 3
2. Hymenial surface turns purplish on putting a drop of 3% KOH solution, basidiospores 3.5–4.75 × 2.5–3.0 µm ..... *P. himalayensis*
2. Hymenial surface not changing color in 3% KOH solution, basidiospores 5.0–7.1 × 2.5–3.8 µm ..... *P. mussooriensis*
3. Basidiospores broadly ellipsoid, 5–6 × 3.5–4.5 µm ..... *P. darjeelingensis*
3. Basidiospores oblong-narrowly ellipsoid-sub cylindrical 4
4. Subiculum well developed ..... *P. gigantea*
4. Subiculum scanty or lacking ..... *P. roumeguerei*

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Pseudocolus fusiformis, an uncommon stinkhorn new to Turkish mycobiota

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Abstract *Pseudocolus fusiformis* is the first representative of the genus to be recorded from Turkey. The species is characterized by basidiocarps with three apical arms that are distally fused and inner surfaces covered by a dark greenish mucoid gleba. A short description and macro- and microphotographs are provided.

Key words — *Basidiomycota*, biodiversity, new record, *Phallales*

Introduction

Pseudocolus Lloyd is a small and uncommon stinkhorn genus, which includes *P. fusiformis*, which is characterized by white to pale orange stem bearing usually 3-4 apical arms united at the their tips and dark greenish mucoid gleba covering the inner surface of the arms (Hemmes & Desjardin 2002, Meyers 2004, Phillips 2005). According to current checklists (Solak et al. 2007, Sesli & Denchev 2010) and latest records (Uzun et al. 2010, Kaya et al. 2010), the genus has not yet been reported from Turkey. Our recent collection of *P. fusiformis*, therefore, constitutes the first report of the species and genus for Turkey.

Materials & methods

The fungi samples were collected from Yomra district of Trabzon province on the east of Black Sea coast of Turkey. Specimens were photographed, and their morphological and ecological properties were recorded in their natural habitats. Thereafter the samples were taken to the laboratory for further investigation. Some reagents (distilled water, Melzer's reagent, 5% KOH) were used for microscopic investigation. Microphotographs of basidiospores were taken under a light microscope (Leica DM 1000). The specimens were identified based on their macroscopic and microscopic features and aided by the literature (Sumstine 1916, Blanton 1977, Blanton & Burk 1980, Quadraccia 1983, Hemmes & Desjardin 2002, Meyers 2004, Phillips 2005). A short description, images,

habitat, geographical position, locality and collection date of the species are also provided. All specimens were deposited at the herbarium of Ankara University (ANK).

Taxonomy

Pseudocolus fusiformis (E. Fisch.) Lloyd, Syn. Phalloids: 53 (1909). FIG. 1

≡ *Colus fusiformis* E. Fisch., Neue Denkschr. Allg. Schweiz. Ges. Gesammten Naturwiss. 32(1): 64 (1890).
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≡ *Pseudocolus javanicus* (Penz.) Lloyd, Mycol. Notes 2: 358 (1907).
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Pseudocolus rothae E. Fisch. ex Lloyd, Phalloids Australas.: 20 (1907).
"*Colus rothae*" E. Fisch., Neue Denkschr. Allg. Schweiz. Ges. Gesammten Naturwiss. 33(1): 23 (1893), nom. inval.
≡ *Colus rothae* (Lloyd) Sacc. & Traverso, Syll. Fung. 19: 389 (1910).
≡ *Anthurus rothae* (Lloyd) G. Cunn., Proc. R. Soc. N.S.W. 56: 188 (1931).
Colus schellenbergiae Sumst., Mycologia 8: 183 (1916).
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Pseudocolus jaczewskii Woronow, Izv. Kavkaz. Muz. 11: 196 (1918).

IMMATURE FRUIT BODY 12–16 mm broad, globose to subglobose, resembling pear to egg shaped; peridium white to whitish gray or pale gray, thin, smooth, opening irregularly along three seams; rhizomorphs whitish, filiform, attached to the base. MATURE FRUIT BODY 40–70 mm height, forming a stalk with tapering three united arms and a volva, arms 2–4 times length of stipe. Stalk white to whitish gray or pale gray, 15–30 mm height, 5–20 mm wide, not extending past volva, thin-walled, hollow, chambered, transversely rugose, flaring toward the upper end. ARMS are three in all samples, bright orange to red orange, lanceolate, spongy, united at apex, cylindrical to flattened, transversely rugose and chambered. GLEBA olive-green to dark green and slimy. VOLVA white to whitish gray or grayish, rugose and tough.

BASIDIOSPORES 2.8–3.2 × 4.9–5.6 µm, ellipsoid, smooth, hyaline. BASIDIA with 6–8 spores.

ECOLOGY solitary or scattered, on disturbed soil in deciduous, coniferous, or mixed forests, gardens; also on old woodchip mulch, thick grass, leaf mulch and humus.

SPECIMEN EXAMINED: **TURKEY**—TRABZON: Yomra, İkisu Village: in common hazel (*Corylus avellana*) garden, in soil, 40°56′30″N - 39°48′55″E, 84 m, 21 August 2009, leg. Akata and Doğan, Akata 2141.

Discussion

Pseudocolus fusiformis is characterized by its onion shaped fruiting body when young and 3–4 (occasionally up to 6) arms when mature (Blanton & Burk 1980). From our measurements taken from 15 fresh specimens, the radius of the four unopened onion shaped fruiting bodies averaged 12–16 mm. All

FIGURE 1. *Pseudocolus fusiformis*: a,b. mature fruit body; c. immature fruit body; d. spores.

specimens bore only three arms. Mature fruit bodies measured 40–70 × 12–30 mm, and the lengths of the arms were 34–57 mm. These results are confirmed in Blanton & Burk (1980) and Quadraccia (1983).

In the literature, basidiospore sizes for *P. fusiformis* are reported as 1.7–2 × 4.5–5 µm by Burk (1976), 1.8–2 × 3.2–5.5 µm by Quadraccia (1983), and 1.5–2 × 3.75–4.5 µm by Blanton (1977). The spore sizes in our collection —2.8–3.2 × 4.9–5.6 µm — are greater than those given in the relevant literature.

This species appears not to require a specific habitat. *Pseudocolus fusiformis* occurs in habitats possessing mulch, wood mulch, or humus, and has been reported from Africa (La Réunion), Asia, Australia, Europe, North America, and South America (Blanton & Burk 1980).

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We would like to thank Mitko Karadalev, Abdullah Kaya, Gabriel Moreno, and Shaun Pennycook for reviewing this article.

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New lichen records from Turkey – 2: *Aspicilia, Protoparmeliopsis,* and *Ramalina*

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Abstract — *Lecanora albosparsa* is lectotypified, the new combination *Protoparmeliopsis klauskalbii* is proposed, and three species of lichen-forming fungi — *Aspicilia albosparsa, P. klauskalbii,* and *Ramalina carpatica* — are reported as new to the lichen biota of Turkey

Key words — Afyon, *Ascomycetes*, Batman, Diyarbakır, Elazığ, İzmir, Şırnak, Uşak

Introduction

Examination of last century collections from Turkey in the herbaria of Vienna (W, WU) has revealed *Aspicilia albosparsa* and *Protoparmeliopsis klauskalbii* as species new to the Turkish lichen biota. These species and *Ramalina carpatica* have also been recently collected by the authors.

Materials & methods

Specimens deposited in W and WU were studied in Vienna, while the type specimens of *Aspicilia albosparsa*, deposited in BC, were studied in Barcelona. Fresh materials were collected from Afyon and Uşak provinces in 2009 and 2010 and deposited at the Aegean University Herbarium (EGE).

The samples were identified using a stereomicroscope, a compound microscope, and standard spot tests. Measurements were made on dry herbarium material or in microscopic preparations in tap water. Hand sections were mounted in water or in lactophenol-cotton-blue (Merck Rf. 13741). To study the ascus structure, Lugol's solution was added to sections and squash preparations after pre-treatment with 10% KOH.

The descriptions presented below are based on both original descriptions (Fritze & Ilse 1870, Sipman 2007, Werner 1958) and our own observations.

For secondary metabolite study, high performance thin-layer chromatography (HPTLC) was performed in accordance with Arup et al. (1993), using a CAMAG Nanomat 4 sample applicator and CAMAG Horizontal Development Chamber.

Taxonomy

Aspicilia albosparsa (Werner) S.Y. Kondr., Ukr. bot. Zh. 59: 603 (2002) FIG. 1

≡ *Lecanora albosparsa* Werner, Bull. Soc. Bot. France 105: 241 (1958).

TYPUS: Syrie, roches volcaniques du littoral près Banias, 27.8.1938, *R.G. Werner* (BC!-**lectotypus, hic designatus**; BC!-isolectotypus).

KEY CHARACTERS— Thallus usually sterile, epilithic, crustose (becoming semi-umbilicate occasionally) and squamulose, with dirty green to ochraceous brown colours, with dominancy of white pseudocyphellae on the surface; hymenium rather shallow to medium, up to 125 µm, with non-moniliform paraphyses; conidia rather short (6–9 µm); secondary metabolites absent.

DESCRIPTION— Thallus epilithic, crustose, sometimes becoming semi-umbilicate (only seen in the collection locality from Uşak!), areolate-effigurate, marginal lobes up to 2 mm long and slightly ascending, flat to slightly convex, dirty green to ochraceous brown, epruinose; pseudocyphellae dominating the surface, white, 1 to 8 in each areole, at first punctiform than becoming elongated and joining into sinuous, branched lines; cortex unistratose, paraplectenchymatous, constructed of perpendicular and moniliform hyphae, up to 50 µm tall, outer part up to 25 µm brown; soredia and isidia absent; prothallus not visible. Ascomata rare, with a black disc, at first punctiform and concave, then slightly convex and up to 1 mm in diam.; excipulum hyaline, up to 30 µm broad, I–; hymenium 75–125 µm tall, hyaline, I+ blue; epihymenium *Aspicilia*-green, N+ green to blue-green; hypothecium distinct, shallow, to 40 µm tall, hyaline, without algae, I–. Asci clavate, *Aspicilia*-type, 75–85 × 19–25 µm, usually sterile; paraphyses septate, simple or branched, 2.5 µm broad, apical cells swollen, up to 3.8 µm; mature ascospores not seen. Conidia straight or slightly curved, 6–9 × 1.3 µm.

SECONDARY METABOLITES— None detected by HPTLC. Spot tests K–, C–, KC–, Pd–.

DISTRIBUTION AND ECOLOGY— This xerophytic species, known from Syria and Turkey (FIG. 2), is perhaps common in the Eastern Mediterranean. It grows on sun-exposed calcareous rocks up to 1700 m altitude in the eu-mediterranean to the mediterranean-montane belt.

ADDITIONAL SPECIMENS EXAMINED **AFYON**, BETWEEN ÇAĞLAYAN & KEKLICEK VILLAGES, 1st km, Kıraç Mountain, 38°10.26'N, 30°10.48'E, 1140 m, 25 June 2009, *A. Şenkardeşler* (*EGE 39726*); BETWEEN MURATLI & KEKLICEK VILLAGES, 1st km, Kıraç Mountain, 38°10.10'N, 30°08.67'E, 1490 m, 25 June 2009, *A. Şenkardeşler* (*EGE 39728*); BETWEEN PAZARAĞAÇ & İNLI VILLAGES, 5th km, 38°31.80'N, 30°49.38'E, 1060 m, 25 May 2010, *A. Şenkardeşler* (*EGE 39728*); **BATMAN**, "in valle Sassun districtus Bitlis, ad rupes prope vicum Goro" [on rock probably in environs of Sarıyayla village], 1700 m, 12 Aug. 1910, *H.F. von Handel-Mazzetti* Nr. 3305 (*W 1929-15489*); **ELAZIĞ**, near GÖLDSCHIK (GÖLCÜK) VILLAGE, at subsaline lake with same name (source of western Tigris), 1350 m, 28 July 1910, *H.F. von Handel-Mazzetti* Nr. 2543 (*W 1920-00704*); **ŞIRNAK**, DSCHESIRET-IBN-OMAR (Cizre), ca. 400 m, 21 Aug. 1910, *H.F. von Handel-Mazzetti*

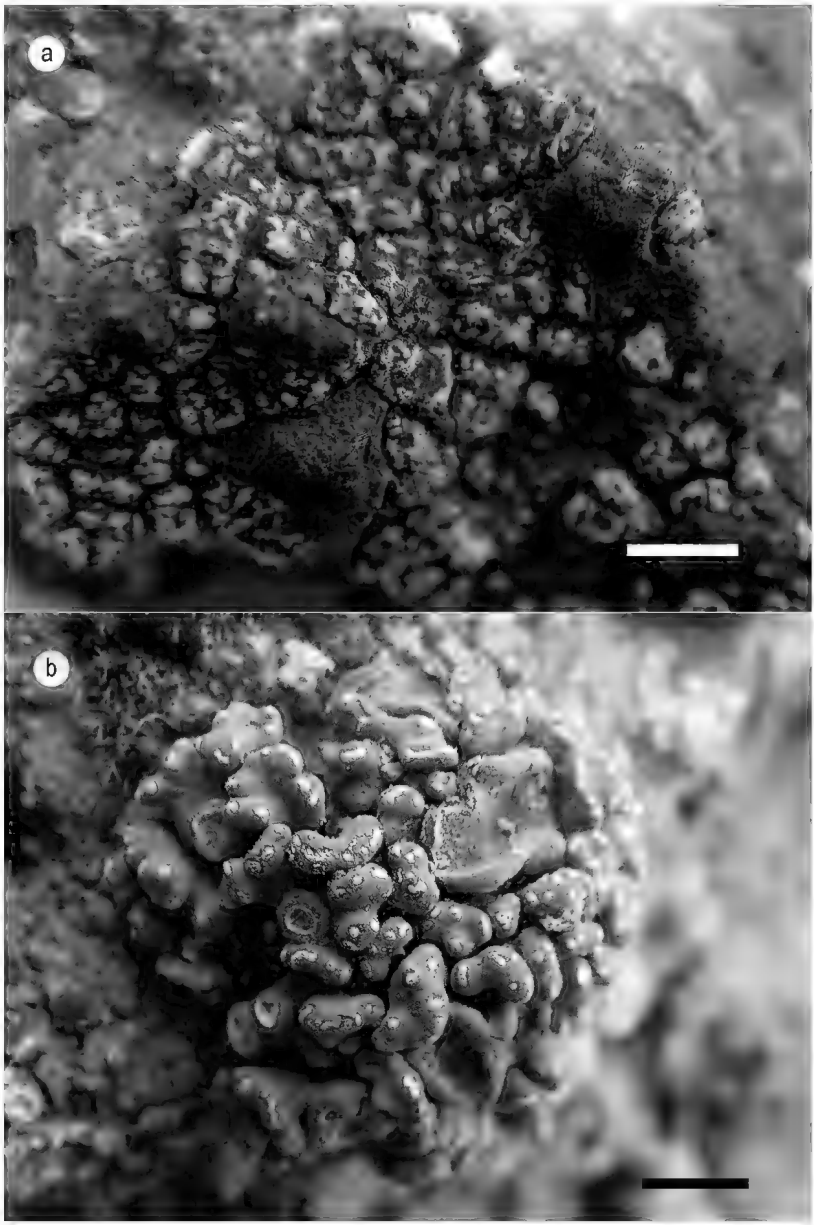

FIGURE 1: *Aspicilia albosparsa*. (a) Lectotype: white, punctiform to jointed pseudocyphellae dominate the surface of the crustose thalli (*BC*); (b) Semi-umbilicate form with mainly punctiform pseudocyphellae (*EGE 39729*); scale bars = 2 mm.

Nr. 3082 (*W 1929-15643*, *WU 41317*); Uşak, between Ormandami & Üçkuyular villages, 3rd km, 38°47.28' N, 29°06.24' E, 920 m, 22 Apr. 2010, *A. Şenkardeşler* (*EGE 39730*).

Remarks— (1) *Aspicilia albosparsa*, which is not included in any identification key, is characterized by a usually sterile, epilithic, crustose (occasionally becoming semi-umbilicate) and areolate-effigurate thallus with dirty green to ochraceous brown colours, a surface largely covered by conspicuous white pseudocyphellae, a rather shallow to medium (≤ 125 µm thick) hymenium with non-moniliform paraphyses, rather short conidia (6–9 µm), and the absence of secondary metabolites.

(2) The species is morphologically similar to *A. maculata* (H. Magn.) Oxner, *A. sphaerothallina* (J. Steiner) Szatala, and *A. syriaca* (J. Steiner) Szatala. *Aspicilia albosparsa* differs from these species by one or more of the following characters: white pseudocyphellae, shorter conidia (8–12 µm in *A. maculata*, 7–13 µm in *A. sphaerothallina*, and 8–15 µm in *A. syriaca*) and a shallower hymenium (100–120 µm in *A. maculata*, 140–190 µm in *A. sphaerothallina*, and 140–180 µm in *A. syriaca*).

(3) Specimens seen in the Natural History Museum in Vienna (W) and the University of Vienna Herbarium (WU) in 2007 and 2008 were labeled "*Lecanora (Aspicilia) pseudocyphellata* J. Steiner", a perfect name reflecting its main key character but one that was never published. In 2009 the author found a suitable type in the herbarium of Guy Roger Werner (BC), labeled as *Lecanora albosparsa*, which was later transferred to *Aspicilia* in Kondratyuk & Zelenko (2002).

(4) The localities of the specimens collected by H.F. von Handel-Mazzetti cause problems, because many village names are no longer in use or unusually spelt. A map deposited in W provided some information about these localities. The locality "Goro" lies about 50 km east of Bitlis province; this information directs the author to the surroundings of Sarıyayla village within Batman province. The locality "Göldschik" corresponds to the present village Gölcük in Elazığ province; the lake indicated in the label is currently called Hazar Lake, lying just east of Gölcük. Dschesiret-ibn-Omar is the former name of Cizre village in Şırnak province.

Protoparmeliopsis klauskalbii (Sipman) Şenkard., **comb. nov.** Fig. 3

MycoBank MB 518655

≡ *Lecanora klauskalbii* Sipman, Biblioth. Lichenol. 96: 270 (2007).

Typus: Greece, W Aegean, Nomos Evvias, Eparchia Karistias, S Evvia, Aetós, alt. ca. 20 m, schist rock outcrops along the coast and in phrygana, 23.09.2005 H. Sipman & Th. Raus no 54654 [B-holotype].

Key Characters— Thallus placodioid, brown, without pruina; areoles with white margins and non-swelling lobes with black lower side; apothecia with

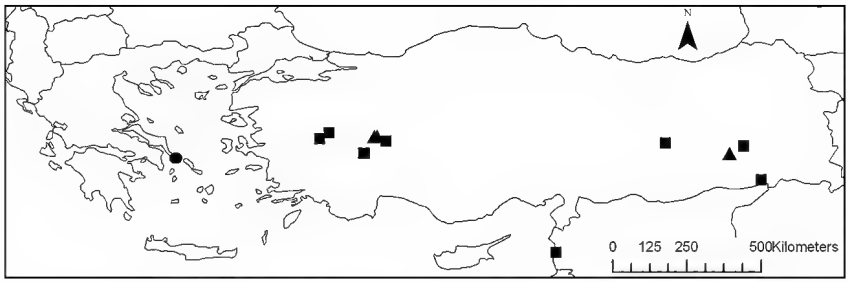

FIGURE 2. Distribution of *Aspicilia albosparsa* (■) and *Protoparmeliopsis klauskalbii* (▲ Specimens examined, ● Locality cited in Sipman 2007)

brown discs; lecanoric acid present (in Turkish specimens) (C+ orange to reddish), usnic and norstictic acids absent (K–, KC–).

DESCRIPTION— Provided by Sipman (2007).

SECONDARY METABOLITES— Zeorin, lecanoric acid and unknown fatty acids. Spot tests K–, C+ orange to reddish, KC+ orange to reddish, Pd–.

DISTRIBUTION AND ECOLOGY— This species, known from the Aegean island of Evvia (Greece) and three localities in the western and eastern central highlands of Turkey (FIG. 2), is probably widespread throughout the eastern Mediterranean.

SPECIMENS EXAMINED **AFYON**, between ATAKÖY & KÜÇÜKKALECIK VILLAGES, 2nd km, Kumalar Mountains, 38°40.99' N, 30°32.78' E, 1140 m, 23 June 2009, *A. Şenkardeşler* (*EGE 39731*); between BÜYÜKKALECIK & KOCATEPE VILLAGES, 3rd km, Kumalar Mountains, 38°40.06' N, 30°28.30' E, 1700 m, 23 June 2009, *A. Şenkardeşler* (*EGE 39732*). **DIYARBAKIR,** near GORO VILLAGE [close to ÇATAKKÖPRÜ VILLAGE] in Sassun valley, ca. 700 m, 08 Aug. 1910, *H.F. von Handel-Mazzetti* Nr. 3308 (*WU 41350*, labelled as *Lecanora riparia*, published in Steiner (1921); specimen attached with a note of Bruce Ryan in 1992 as "Holotype of *Lecanora steineri* ined."; *W 1929-15537*, labelled as *Lecanora riparia*, published in Steiner (1921); specimen annotated by Bruce Ryan in 1992 as "Isotype of *Lecanora steineri* ined.").

REMARKS— (1) *Protoparmeliopsis klauskalbii*, not yet included in any identification key, differs from *Lecanora graeca* J. Steiner and *L. placentiformis* J. Steiner by the absence of norstictic acid and from other placodioid species of *Lecanora* Ach. and *Protoparmeliopsis* M. Choisy by the absence of usnic acid and the presence of lecanoric acid. It differs from related species known from Turkey and adjacent regions by the combination of a brown thallus with white areole margins, non-swelling lobes, and a black underside. *Lecanora bolcana* (Pollini) Poelt, *L. dispersoareolata* (Schaer.) Lamy, *L. garovaglii* (Körb.) Zahlbr., *L. graeca*, *Protoparmeliopsis achariana* (A.L. Sm.) Moberg & R. Sant., *P. laatokkaensis* (Räsänen) Moberg & R. Sant., *P. macrocyclos* (H. Magn.) Moberg & R. Sant., and *P. muralis* (Schreb.) M. Choisy are not brown and their areoles lack white margins; unfortunately, old herbarium material of these 8 species

FIGURE 3: *Protoparmeliopsis klauskalbii*. Thallus margin (W *1929-15537*); scale bar = 2 mm.

often becomes brownish, so that TLC/HPTLC analyses become necessary to differentiate them from *P. klauskalbii*. *Lecanora placentiformis* is also brown but its areolar margins are not white.

(2) The Turkish material seems to deviate by the presence of lecanoric acid, a substance not reported by Sipman (2007).

(3) Choisy (1929) briefly described the genus *Protoparmeliopsis* as having a placodioid and submembranous thallus with hypothecium containing photobionts ["Thalle submembraneux, lacinié divisé au pourtour, straminé, apothécies vulgairement pénétrées par les gonidies dans l'hypothécium."]" and as type species proposed *P. muralis*, which has long (≤ 25 µm), curved pycnoconidia. Hafellner & Türk (2001) and Santesson et al. (2004) have recently proposed many new combinations, and the genus has been accepted in numerous local floristic studies. According to Sipman (2007), *P. klauskalbii* is closely related to *P. muralis* and fits well in *Protoparmeliopsis* even in a narrower concept of the genus. Therefore we do not hesitate to propose this new combination even in the absence of one generic character—the straw-like colour (presence of usnic acid). Other transfers to *Protoparmeliopsis* will be made in future papers.

(4) Steiner used the name *Lecanora riparia* (Flot.) J. Steiner for specimens related to "*Lecanora muralis*" with a brown colour and whitish margins,

conspecific with *P. klauskalbii* (specimens seen by the authors in W and WU). However, this name is not applicable because the type specimen is from Central Europe, where *P. klauskalbii* does not occur. Bruce Ryan, who reinvestigated these specimens using TLC and annotated some as "*Lecanora steineri* B. Ryan ined.," died before he could publish his species. Later, Sipman (2007) realized that the usnic acid-lacking, brown, white-margined specimens collected in Greece should be treated as a distinct species, for which he proposed the name *Lecanora klauskalbii*.

(5) As mentioned above, recognition of the collection localities of H.F. von Handel-Mazzetti is problematic. The map deposited in W situates "Batman Köprü" about 15 km east of Mejafarkin (currently known as Silvan). It is inside the province of Diyarbakır, close to the border with Batman, a location that matches the current environs of Çatakköprü.

Ramalina carpatica Körb., in Fritze & Ilse, Verh. Zool.-Bot. Ges. Wien 20: 501 (1870).

TYPE: "Mittelgrat", Vysoké Tatry, Slovakia, 1863, leg. Hausknecht & Fritze [W? not seen].

KEY CHARACTERS— Thallus epilithic, erect, with distinctly blackened branch tips, lacking soredia, isidia and pseudocyphellae; apothecia apical, occasionally blackening like the tips of branches; ascospores kidney-shaped.

DESCRIPTION— Thallus epilithic, shrubby, tufted, erect, up to 9 cm high; branches usually single, thick, smooth to unevenly pitted, hollow, cylindrical or rarely flattened at the base, not dorsiventral and not filamentous; soredia, isidia and pseudocyphellae absent; tips of the branches distinctly blackened; cortex plectenchymatous of perpendicular hypes, thin, indistinct, overlaying a strongly developed and cylindrical zone of prosoplectenchymatous tissue including the photobiont zone; photobiont trebouxioid. Ascomata apothecia, frequent, apical, partly blackened, shortly stalked, disc pale green or blackened. Asci elongate-clavate, *Bacidia*-type, 8-spored. Ascospores 1-septate, kidney-shaped, colourless, 11–16 × 5–7 µm.

SECONDARY METABOLITES— None detected by HPTLC. Spot tests K–, C–, KC–, Pd–. Earlier studies report evernic, obtusatic and usnic acid for this species (Culberson 1970). Our material seems to belong to a deficient strain.

DISTRIBUTION AND ECOLOGY— This species, previously known only from Poland to Greece in the Carpathian Mountains and the Balkans (Keissler 1959, Abbott 2009), is reported here for the first time from Turkey. It is a saxicolous species from wind-exposed cliffs of acidic silicate rocks.

SPECIMEN EXAMINED— **İZMIR**, Bozdağ Mountains, between YILANLI & DOKUZLAR VILLAGES, 1st km, 38°18.22' N, 28°07.45' E, 2063 m, 11 May 2009, *S. G. Şenol*, conf. O. Blum (*EGE 39733*).

REMARKS— (1) *Ramalina carpatica* is easily recognizable at first glance by its habit on siliceous rocks and its blackening tips and apothecia. The collected specimens, reaching up to 9 cm tall, are the longest reported for this species.

(2) The identification of this species was confirmed by Prof. Dr Oleg Blum (Kiev).

Acknowledgements

I thank Dr László Lőkös (Budapest) and Dr Harrie J.M Sipman (Berlin) for reviewing this paper. Dr Harrie J.M Sipman (Berlin) is acknowledged for his useful comments on *Protoparmeliopsis klauskalbii*, Dr Serdar G. Şenol (Izmir) for collecting *Ramalina carpatica* and Dr Oleg Blum (Kiev) for the confirmation of *R. carpatica*. The study in the Institut Botànic de Barcelona was supported by the Ege University (09 FEN 055), that in the Herbarium of the Natural History Museum Vienna was financed by the mobility program of The Scientific and Technological Research Council of Turkey (TUBITAK), that in the University of Vienna Herbarium was awarded by the Synthesys Project (http://www.synthesys.info/) which is financed by the European Community Research Infrastructure Action under the FP6 "Structuring the European Research Area" Programme, while the specimens from Afyon and Uşak provinces were collected in the framework of the TUBITAK Research Project No 106T628 and by the Ege University (08 BIL 025).

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Tropical *Trametes lactinea* is widely distributed in the eastern USA

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Abstract *Trametes lactinea* is reported from the USA for the first time. This large and conspicuous polypore, known until now only from tropical regions, was collected in many different localities in eastern USA. ITS region of six collected specimens and four others from public and private herbaria was sequenced and compared with the sequences in the GenBank. Important macro- and micro-characteristics that discriminate between *T. lactinea* and its similar species *T. elegans* are pointed out and discussed.

Key words *Basidiomycota*, USA new record, taxonomy, invasive species

Introduction

Since 2001, we have occasionally collected in the eastern USA (from Virginia to Connecticut) a very large (≤ 7 cm thick, ≤ 20 cm wide), white to light greyish *Trametes* species with a velutinous but not hirsute pileus and large, regular pores that appear a bit brownish when old. The collections, which were always sterile, could not be identified using the keys for USA *Trametes* species. In early spring 2009 when travelling around southern Florida we noted a great abundance of a very similar, but thinner and fertile, *Trametes* sp. that was in many localities the dominant fungus on both hardwoods and pines. In this article we present evidence that both the early spring/southern and the autumn/northern USA collections represent the same species, *Trametes lactinea*, that is, accordingly, widely distributed in the eastern USA and locally very common, in contrast to literature data.

Trametes lactinea is a typical *Trametes* species with a white tough context, white tomentose pileus, and large pores. Described from Sri Lanka (Berkeley 1843), it was reported as common in the tropics of East Asia and Australia, rare

TABLE 1. *Trametes lactinea* and *T. elegans* specimens examined.

SPECIMEN	*	LOCALITY	SUBSTRATE	COLLECTED
T. lactinea				
PRM560783	1	Fungi of west Pakistan 15286, coll. S. Ahmad	*Morus alba*	27.II.1961
Damm 8936	**2**	Mahé, Beau Vallon, Seychelles	*Cocos*	10.II.2008
Damm 4703	**3**	Mauritius Island	hardwood	13.II.2000
JV0108/8 H		Valley Forge, PA, USA	*Populus*	1.VIII.2001
JV0309/130 J		Hatfield Apartments, Hatfield, PA, USA	*Carya*	18.IX.2003
JV0309/186A	4	Promised Land St. Park, Pike County, PA, USA	beech	22.IX.2003
JV0312/28.2 J		Tree Tops Park, Miami, FL, USA	*Casuarina*	28.XII.2003
JV0410/11 J	5	Mason Neck St. Park, Woodbridge, VA, USA	*Nyssa*	9.X.2004
JV0709/179		Mason Neck St. Park, Woodbridge, VA, USA	hardwood	22.IX.2007
JV0808/29	6	Swamp Creek, Schwenksville, PA, USA	oak	28.VIII.2008
JV0809/50	7	Yale Univ. arboretum, New Haven, CN, USA	beech	13.IX.2008
JV0904/23		Royal Palm, Everglades N.P., FL, USA	hardwood	19.IV.2009
JV0904/24	8	Royal Palm, Everglades N.P., FL, USA	hardwood	19.IV.2009
JV0904/27		Royal Palm, Everglades N.P., FL, USA	hardwood	19.IV.2009
JV0904/43 PRM915660	9	Long Pine Key, Everglades N.P., FL, USA	pine	19.IV.2009
JV0904/79		Long Pine Key, Everglades N.P., FL, USA	pine	21.IV.2009
JV0904/93		Long Pine Key, Everglades N.P., FL, USA	pine	22.IV.2009
JV0904/120		East Main Tram, Fakahatchee Strand Preserve, FL,USA	hardwood	22.IV.2009
JV0904/127		Collier Seminole St. Park, FL, USA	pine	23.IV.2009
JV0409/145 PRM915661	**10**	Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
JV0904/146		Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
JV0904/147		Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
JV0904/148		Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
JV0904/148A		Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
T. elegans				
PRM871961		Esmeralda pr. Camagüey, Mt. California, Cuba	*Eugenia*	14.X.1966
PRM887367	**11**	Pico Perillo, Sierra del Escambray, Cuba Centr.	hardwood	5.I.1967
6069 Dammrich	**12**	Ko Samui, Thailand	hardwood	15.II.2002
JV0212/37 J	**13**	Gainsville, FL, USA	*Carya*	20.XII.2002
JV0411/2 Ja&Ro		Cameroon Highlands, Malaysia	hardwood	27.XI.2004
JV0511/2 Kop		Ecuador	hardwood	3.XI.2005

* Numbers indicate sequenced specimens. Bold numbers indicate specimens with ribosomal RNA ITS region sequence deposited in GenBank: **2** – HM756192, **3** – HM756193, **10** – HM756191, **11** – HM756196, **12** – HM756194, **13** – HM756195. Sequences 1, 4, 6, 7, 8 are identical with published sequence AY684178; sequences 5, 9, **10** are also identical.

in southern China and Africa, but not occurring in tropical America (Corner 1989, Dai 2009, Dai et al. 2004, Ryvarden 1976, Ryvarden & Johansen 1980). Since 2000, its occurrence in South America has slowly been acknowledged (Ryvarden 2000, 2001), but there are no previous records for the USA. We have compared our USA collections with three standard *T. lactinea* samples from public and private herbaria, including ITS region sequencing, and found that they are identical. We also show that other sequences in the GenBank annotated as *T. lactinea* are often derived from another species, *Trametes elegans* (Spreng.) Fr., and so we summarize here the most important characteristics that allow to discriminate these two species. Comments regarding other lenzitoid *Trametes* species are also provided.

Materials & methods

Specimens studied

We collected 13 fertile specimens of *T. lactinea* in early spring 2009 in Florida and nine other sterile specimens from various states of eastern USA from late summer through early winter during 2001, 2003, 2004, 2007, and 2008. We also studied one sample (unfortunately sterile) of *T. lactinea* obtained from PRM collection in Prague and one fertile sample from Frank Dammrich private collection that was reputedly determined by Prof. Ryvarden. We have also inspected some typical samples of similar *T. elegans* (Table 1) and one aberrant sample from the Dammrich collection labeled as *T. menziesii* (Berk.) Ryvarden. Pieces of dried basidiocarps mounted in water were examined microscopically and 20 basidiospores from each specimen were measured. One drop of 10% KOH was added to study the swelling of skeletal hyphae. Specimens are deposited in the private herbarium of the first author (http://mykoweb.prf.jcu.cz /polypores) and PRM (Table 1).

ITS amplification and sequencing

0.25 g of the context tissue from dried specimens was frozen and disintegrated 60 s with steel ball in mixer mill MM301 RETSCH under liquid nitrogen. DNA was isolated using CTAB/NaCl extraction buffer as described by Murray & Thompson (1980), followed by repeated extraction with chloroform and isopropanol precipitation. Crude DNA was dissolved in 100 µl of sterile water and further purified using Wizard Clean Up kit PROMEGA. Resulting DNA solution (50 µl) was diluted ten times and 1 µl was used as template for amplification with ITS1 and ITS4 primers (White et al. 1990) in 25 µl reaction mixture using 55°C annealing temperature. Amplified DNA was purified using Wizard Clean Up kit PROMEGA and sequenced in the Genomics laboratory of Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, on ABI 3730xl DNA analyzer, using BigDye Terminator 3.1 kit.

ITS sequence analysis

The sequences were aligned by Clustal X and manually pruned. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004). All positions containing gaps and missing data were

eliminated from the dataset (complete deletion option). There were a total of 454 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Taxonomy

Ryvarden & Johansen (1980) and Corner (1989) published very good descriptions of *Trametes lactinea;* here we compile the most important features and add some new characteristics based on our own observations of many fresh USA specimens.

Trametes lactinea (Berk.) Sacc. FIGURE 1

Photos in (http://mykoweb.prf.jcu.cz/polypores)

BASIDIOMES annual to biennial, solitary to more rarely imbricate (when growing on short stumps), pileate, broadly to narrowly attached, 1–25 cm broad and wide, and 0.5–2 cm thick (exceptionally up to 7 cm thick at the base in specimens from the Northern USA), hard corky; PILEUS semicircular, mostly applanate but sometimes rather thick at the base, surface velvety, but with no hairs visible under 20× lens, becoming warted with age, sometimes with irregular outgrows especially near the base, azonate or very slightly concentrically sulcate, sometimes with narrow greyish zones near the margin or with wide, concentric furrows that mark periods of an intensive growth; MARGIN obtuse and thick at first, sterile below, later sharpened, with pores developed to the very edge; PORES at first white, later cream to ochraceous and darker than the upper surface, at first round, thick-walled, later with wedge-narrowed edges, in old specimens thin-walled, distinctly angular, but never labyrinthine, 2–3 per mm; TUBES concolorous with the context, 6–12 mm long; CONTEXT 5–20 mm, sometimes up to 60 mm thick, white, corky.

Hyphal system trimitic, GENERATIVE HYPHAE clamped and thin-walled, 1–3 μm in diam.; SKELETAL HYPHAE abundant, hyaline, mostly with only moderately thickened walls, 4–6 μm in diam., but sometimes 3–4 μm broad and almost solid. After adding 10% KOH the solid hyphae swell to 10–15 μm in diam. and shrink quickly in vivid movements; in moderately thickened hyphae the swelled walls only fill the hole but the outer diameter remains unchanged. BINDING HYPHAE abundant, hyaline, thick-walled, arboriform, 1–5 μm in diam.; BASIDIA clavate, 4-sterigmate, 12–16 × 5–7 μm; CYSTIDIA none; BASIDIOSPORES oblong-ellipsoid to cylindric, hyaline, negative in Melzer's reagent, 5–7 × 2.5–3.2 μm, thin-walled.

Differentiation between *Trametes lactinea* and *Trametes elegans*

Because *Trametes lactinea* keys in the American literature as *T. elegans* (Gilbertson & Ryvarden 1987), we summarize here the most important characteristics of both species. Spores are clearly diagnostic — they are distinctly

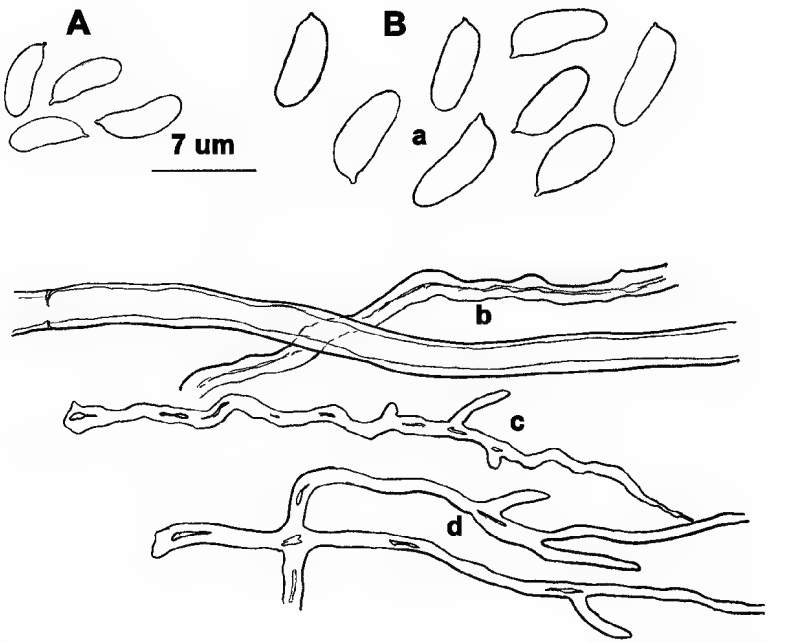

FIGURE 1: *Trametes elegans* PRM 887367 (A): basidiospores. *Trametes lactinea* (B): a. basidiospores, b. skeletal hyphae, c, d. binding hyphae.

larger in *T. lactinea* (usually > 6 μm × 3 μm) and more curved and smaller (< 6 μm × 2.5 μm) in *T. elegans* (FIG. 1). Basidia of *T. elegans* are also quite small, only 8–13 × 4–6 μm. Unfortunately, many collections of both species are completely sterile. *Trametes lactinea* does not have hyphal pegs but all 6 studied specimens of *T. elegans* had pegs in the tubes that could be best seen on cut tubes under lens as scattered warts or protuberances on tube sides. Although this feature has never been mentioned in *T. elegans* descriptions, we regard it as rather constant. Pores of *T. lactinea* change in the course of basidiocarp development from thick-walled, round, and white to relatively thin-walled, angular, and brownish, but they are never labyrinthine, whereas in *T. elegans* they are mostly lamellate and when poroid, they are a bit elongated or labyrinthine at least in part of the fruitbody. The KOH reaction is only moderate in *T. elegans*: the hyphae swell slightly, without shrinking accompanied by quick hyphal movements. Unfortunately, the typical swelling reaction of *T. lactinea* skeletal hyphae is much weaker or less striking in very old herbarium specimens. The pileus surface of *T. lactinea* is quite variable but never glabrous, as it appears in most specimens of *T. elegans* (and never hirsute like by *T. gibbosa* (Pers.) Fr.).

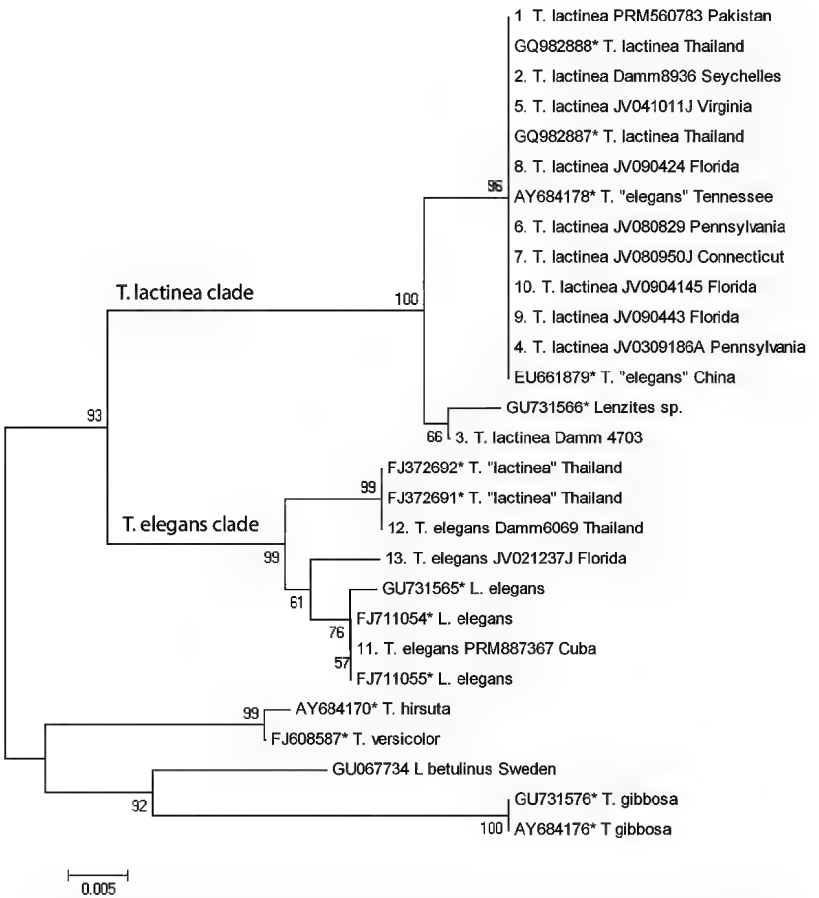

FIGURE 2: Evolutionary relationships of 28 *Trametes* species based on ITS region sequence. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances are in the units of the number of base substitutions per site. Accession numbers with * represent sequences retrieved from GenBank. Other GenBank accession numbers are in TABLE 1.

ITS sequence analysis

All our collections and one PRM herbarium specimen from Pakistan showed striking sequence similarities with only one variable site in ITS1. The sequence is identical with two recently published sequences (GQ982887, GQ982888) of

Trametes lactinea from Thailand and also with sequences AY684178 (Tennessee; Tomšovský et al. 2006) and EU661879 (China, unpublished) that were derived from putative *T. elegans* collections (Fig. 2). The GenBank sequence GU731566, identified as "*Lenzites* sp." and derived from the CIRM-BRFM 1050 strain and also somewhat aberrant specimen of F. Dammrich from Mauritius show about 15 transitions/transversions in ITS region, but overall sequence similarity with *T. lactinea* is prevails (see discussion). Other *T. elegans/lactinea* sequences from GenBank as well as from our own, F. Dammrich, and PRM collections are distinct and comprise a distinctly separate clade (Fig. 2) that represents the true *T. elegans*.

Discussion

The massive fruiting of a previously unrecognized, large polypore, *Trametes lactinea*, in southern Florida is very surprising. It is possible that the species has been misdetermined as *T. elegans* or *T. menziesii* and that many collections in American herbaria are in fact *T. lactinea*. We have not systematically studied its ecology, but it is worth mentioning that during September 2010 in our best localities around Miami, FL, we could not find any basidiocarps except for some disintegrated remnants. We infer that in the southernmost USA, the species develops very late in autumn, survives winter, and disintegrates in spring, which might contribute to its imperceptibility in the past. Nevertheless, the striking sequence homogeneity of all American collections of *T. lactinea* also suggests a relatively recent colonization of the American continent. We have previously described a similar case of the sudden appearance of the European *T. gibbosa* in the northern USA and Canada (Kout & Vlasák 2007); currently, *T. gibbosa* is the dominant polypore in the region, at least in Pennsylvania (Vlasák, unpublished observations 2010). Notably, *T. gibbosa* also had been misdetermined as *T. elegans*. More extensive studies are needed to confirm that *T. lactinea* is a recent invasive species in the USA. Long-term and population studies may also help explain the unexpected spread of *T. lactinea* to northern USA that has, to our knowledge, no parallel in its primary distribution area in Asia.

A somewhat aberrant specimen from Mauritius, Damm 4703, with a small pileus and slightly labyrinthine or split tubes and initially determined as *T. menziesii,* has a sequence that corresponds to most of that from *T. lactinea* but contains several mutations that are often identical with GenBank GU731566 derived from a *Lenzites* sp. collected in French Guiana. These two sequences cluster together with 66% bootstrap support (Fig. 2). The contributors of GU731566 note that several other French Guianan *Lenzites* sp. specimens with quite variable pore surfaces (poroid with 1-2 mm wide pores, or daedaloid, or lamellate) but with similar sequences were all sterile (A. Favel, INRA, Marseille, pers. comm.). Fertile basidiocarps are needed to solve this problem. *Trametes*

menziesii is a similar but variable paleotropical species with no sequence yet deposited in GenBank. According to Ryvarden & Johansen (1980), it should have rather narrow spores, only 1–2 µm wide. We have seen only one specimen with older name *Polyporus meleagris* Berk. in PRM herbarium. It was a very different fungus and so much damaged that we could not isolate DNA.

The *T. elegans* clade (Fig. 2) is much more divergent than that of *T. lactinea* and the division into two branches is well supported. Again, we have no good explanation for it at present. *Trametes elegans* is often regarded a member of genus *Lenzites* (*L. elegans* (Spreng.) Pat.) based on its sometimes lamellate hymenophore and some pointed ends of binding hyphae protruding into hymenium, reminiscent of the catahymenium characteristic of *L. betulinus* (L.) Fr. (Nuñez & Ryvarden 2001). Nevertheless, our ITS region sequence phylogram shows no similarity between *T. elegans* and *L. betulina,* and so we find the name *T. elegans* more appropriate.

Acknowledgements

We are grateful to Josef Vlasák Jr. for collecting of many interesting polypore specimens and revising the English language. We also thank Frank Dammrich for generous loan of his herbarium specimens. Dr. Yu-Cheng Dai and Dr. Stephane Welti have kindly acted as presubmission reviewers and their help is acknowledged. This research was supported by CEZ: AV0Z50510513 fund.

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A new species of *Nephroma* (*Nephromataceae*) from the Tibetan Plateau

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ABSTRACT A new species, *Nephroma flavorhizinatum*, is described from the Tibetan Plateau. This species is characterized by marginal and laminal lobules, an obvious pruina, a white to golden yellow medulla, a golden yellow rhizinal base, and a unique chemistry.

KEY WORDS China, lichen, *Peltigerales*, taxonomy

Introduction

Nephroma Ach. is the only member of the lichen family *Nephromataceae* (*Peltigerales*, *Lecanoromycetes*, *Ascomycota*) (Kirk et al. 2008). The genus is morphologically characterized by a cyanobacterium (*Nostoc*) or a green alga (*Coccomyxa*) photobiont, a coriaceous foliose thallus with cortex on both surfaces, a kidney-shaped apothecium at lobe apices of the lower surface, an 8-spored ascus, and often 3-septate pale brown ascospores (James & White 1987, White & James 1988, Burgaz & Martínez 1999, Brodo et al. 2001, Wetmore & Nash 2002, Louwhoff 2009). Most *Nephroma* species contain a variety of lichen substances, including hopane triterpenoids, phenarctin, usnic acid, perlatolic acid derivatives, anthraquinones, or other pigments (James & White 1987).

Nephroma is a cosmopolitan genus of 36 species worldwide (Kirk et al. 2008). In the southern temperate zone, Australia, North America, Europe, Macaronesia, and South Korea, the species of *Nephroma* were studied systematically (Wetmore 1980, Tønsberg & Holtan-Hartwig 1983, James & White 1987, White & James 1988, Park 1990, Goward & Goffinet 1993, Burgaz & Martínez 1999, Brodo et al. 2001). In China, nine *Nephroma* species were reported: *N. helveticum*, *N. isidiosum*, *N. javanicum*, *N. moeszii*, *N. parile*, *N. resupinatum*, *N. sinense*, *N. subparile*, and *N. tropicum* (Wei 1991).

During our study of the lichen flora of the Tibetan Plateau, an interesting species of *Nephroma* new to science was found.

Materials & methods

The specimens studied were collected from Sichuan, China, and are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University). The morphological and anatomical characters of the specimens were examined using a stereo microscope (COIC XTL7045B2) and a microscope (OLYMPUS CX21). Lichen substances in all specimens cited were identified using the standardized thin layer chromatography techniques (TLC) with G system (Culberson et al. 1981). Photos of the thallus were taken under OLYMPUS SZX12 with DP72.

Taxonomy

Nephroma flavorhizinatum Q. Tian & H.Y. Wang, **sp. nov.** FIGS 1, 2

MycoBank MB 519038

Lobus crenatus. Lobulus marginalis et laminalis. Medulla alba ad flava. Rhizina flava ad basis. Phycobiont Nostoc. Thallus T1, T2 et T4 continens.

TYPE COLLECTION: CHINA. Sichuan province, Litang, alt. 4200 m, on ground, Z. S. Sun, 20080755, 5 Nov. 2008. (**Holotype** in SDNU).

EXPANDED DESCRIPTION —Thallus foliose, coriaceous, about 4 cm in diameter. Lobes irregular, usually 2–10 mm wide, often ascending at the margins, crenulate or with lobules. Upper surface brown, smooth; isidia and soredia absent; lobules marginal and laminal, usually 0.2–1 mm wide, developing from isidioid papillae, immediately becoming flat, some of them branched; pruina white, at the margins of lobes and the dorsal surface of apothecia. Photobiont *Nostoc*. Medulla white to golden yellow. Lower surface dark brown, tomentose, paler and smooth at the margins; rhizines about 3 mm long, brush-like, golden yellow at the bases, white at the tips. Apothecia common, immersed on lower surface at tips of lobes, kidney-shaped, usually erect; disc dark brown, 1–4 mm in diameter; dorsal surface often with short tomentum; margins usually entire, rarely crenulate or with lobules. Asci 8-spored; ascospores pale brown, 3-septate, 15–20 × 3.5–5 μm. Pycnidia not seen.

SPOTS TESTS—Thallus upper surface: K–, C–, KC–, PD–; white medulla: K–, C–, KC–, PD–; golden yellow medulla: K+ violet, C–, KC–, PD–; rhizinal base: K+ violet, C–, KC–, PD–; rhizine tip: K–, C–, KC–, PD–.

SECONDARY METABOLITES—7β-acetoxyhopan-22-ol (T1), 15α-acetoxyhopan-22-ol (T2), hopane-7β,22-diol (T4).

DISTRIBUTION AND SUBSTRATE —*Nephroma flavorhizinatum* is a terricolous species, at present known only from the type locality.

ADDITIONAL SPECIMENS EXAMINED CHINA. Sichuan: Litang. alt. 4200 m, on soil, 5 Nov. 2008, Z.S. SUN 20084011 & 20103317 (SDNU).

FIG. 1 *Nephroma flavorhizinatum* (holotype).
A. Thallus. B. Pruina at the dorsal surface of apothecia. (Scale bars = 2 mm).

COMMENTS—The presence of laminal and marginal lobules, a white to golden yellow medulla, a golden yellow rhizinal base, and the unique chemical characters distinguish *Nephroma flavorhizinatum* from all other *Nephroma*

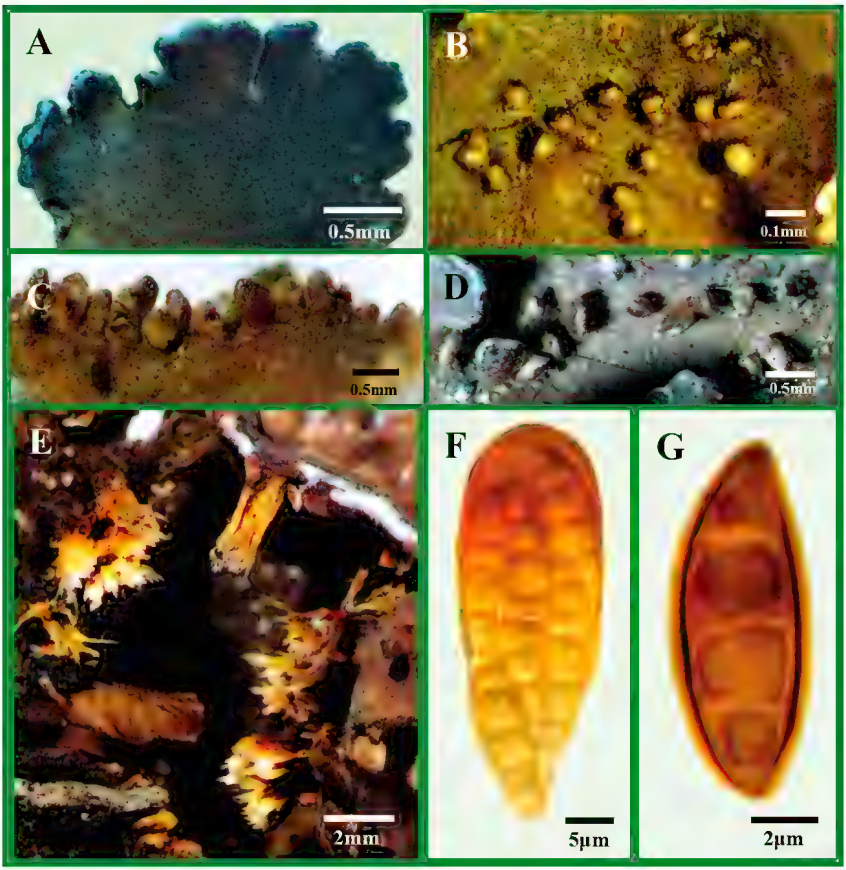

Fig. 2 *Nephroma flavorhizinatum* (holotype).
A. Crenulate lobe. B. Developing laminal lobules. C. Marginal lobules.
D. Developed laminal lobules. E. Rhizines: golden yellow at the bases, and white at the tips.
F. 8-spored ascus. G. 3-septate ascospore.
(Scale bars: A, C, D 0.5 mm; B 0.1 mm; E 2 mm; F 5 μm; G 2 μm)

species. Many *Nephroma* species have laminal lobules (phyllidia or folioles), but most *Nephroma* species have no rhizines. *N. isidiosum*, *N. kuehnemannii* and *N. microphyllum* are rhizinate. However, *N. isidiosum* and *N. kuehnemannii* have no laminal lobules, and the former is isidiate, the latter is lichenized with green algae. *N. microphyllum* has laminal lobules, but its ascospores are 5–8-septate rather than 3-septate, and longer than the new species (70–90 μm vs. 15–20 μm). Moreover, the medulla and rhizines of all the three *Nephroma* species mentioned above are white rather than golden yellow. *N. analogicum*,

N. chubutense, *N. laevigatum*, *N. tangeriense*, and *N. venosum* all have white to yellow medulla and lobules. However, these five species are not rhizinate, and their lichen substances are different from the new species.

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Studies in *Erysiphales* anamorphs (4): species on *Hydrangeaceae* and *Papaveraceae*

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ABSTRACT Anamorphic powdery mildews on *Hydrangeaceae* and *Papaveraceae* in Germany are revised. Species are documented in detail including line drawings, photomicrographs, and identification keys. On *Papaveraceae* three species are accepted, specifically *Erysiphe macleayae* on *Chelidonium majus* and *Macleaya cordata*, *E. cruciferarum* on *Eschscholzia californica*, and *Oidium* sp. (an unknown species previously assigned to *E. cruciferarum*) on *Pseudofumaria lutea*. Species on *Hydrangeaceae* are *Oidium hortensiae* on *Hydrangea macrophylla* and *E. deutziae* on *Deutzia* cf. *scabra* and *Philadelphus* cf. *coronarius*. The fungus on the latter host plant was previously assigned to *O. hortensiae*. *Erysiphe deutziae*, *E. macleayae*, and *Oidium hortensiae* are introduced species.

KEY WORDS — conidial germination, morphology, neomycete

Introduction

In Germany, there are three species of *Erysiphales* reported on *Papaveraceae* (*Erysiphe cruciferarum*, *Erysiphe* cf. *macleayae*, *Golovinomyces orontii* (Castagne) Heluta); and two (*Erysiphe deutziae*, *Oidium hortensiae*) on *Hydrangeaceae* (Braun 1995, Jage et. al. 2010). The following is a revision of anamorphs on certain host plants of *Papaveraceae/Hydrangeaceae (Chelidonium, Deutzia, Hydrangea, Macleaya, Meconopsis, Philadelphus* and *Pseudofumaria)* for which the host/pathogen affiliations have been doubtful.

Materials & methods

Both fresh and dried structures were examined in tap water mounts with light microscopy using Olympus BH 2 and Zeiss Axioskop 2 Plus. Pertinent features were measured at a magnification of 400× or 1000× and documented by line drawings and photographs using a Canon A1 or Canon Powershot A80. From each specimen up to 25 spores were measured. To induce conidial germination, the method of Schmidt & Scholler (2002) was applied. Fresh conidia were spread on a microscope slide and placed

in a Petri dish with moist cellulose tissue. The closed Petri dishes were incubated at room temperature and exposed to daylight through a north-facing window for 24 h.

Specimens were studied from HAL, KR, and private herbaria (Herbarium J. Kruse, A. Schmidt). Specimens collected by the authors were deposited in KR (some duplicates in the private herbarium of the first author). Permanent slides were made using Hoyer's medium (Cunningham 1972). If possible, fresh material was studied, but for study of outer cell wall ornamentation of conidia only dried material was used according to Bélanger et al. (2002).

The species concept and nomenclature follows Braun (1999) and Braun & Takamatsu (2000). The host plants are named according to the Flora Europaea (Tutin et al. 1964–80).

Taxonomic descriptions

Species on *Papaveraceae* (incl. *Fumariaceae*)

Erysiphe macleayae R.Y. Zheng & G.Q. Chen PLATE 1

SPECIMENS STUDIED — On *Macleaya cordata* R. Br.: Germany, Brandenburg, Potsdam-Bornim, Am Raubfang, (MTB 3544/3/3), 8 Nov. 2004, leg. V. Kummer (KR 0021921 and herbarium A. Schmidt no. KM 203) (host species cf.) (fresh); Sachsen, Görlitz, Sonnenstr. 17, front garden of Dunger house of Staatliches Museum für Naturkunde, 25 Oct. 2005, leg. et det. H. Boyle as «cf. *Erysiphe macleayae* R.Y. Zheng & G.Q. Chen» (HAL 2209 F — GLM F070246), with hyperparasite *Ampelomyces quisqualis*; Brandenburg, Teltow, Lichterfelde direction, Lenaustr., 18 Sep. 2009, leg. et det. V. Kummer as «cf. *Erysiphe macleayae* R.Y. Zheng & G.Q. Chen» (HAL 2133 F) (dry). On *Meconopsis cambrica* (L.) Vig.: Germany, Niedersachsen, Hannover, Zoo, Adenauerallee 3, wayside (MTB 3624/2/1), 55 m, 9 Oct. 2009, leg. J. Kruse, det. U. Braun, anamorph + teleomorph with mature ascomata on stem (mixed infection with *Peronospora meconopsidis* Mayor, det. M. Scholler) (herbarium J. Kruse no. E 0293) (dry).

Mycelium conspicuous, dense, either covering the whole surface or around leaf veins, whitish to slightly brownish, amphigenous, but predominantly epiphyllous; sterile hyphae hyaline, branched, smooth, 3.5–7 µm wide; hyphal appressoria lobed or nipple-shaped, singly or in pairs; epiphyllous conidiophores 45–90 µm, 5.5–11 µm wide, attenuated toward base, lowermost cell up 8 µm, uppermost cell up to 11 µm wide, 3-celled, rarely 2- or 4-celled, foot-cell, longest cell of conidiophores, 25–40 µm long, often curved; conidia formed singly, ellipsoid to cylindrical (-doliiform), 26.5–44.0(–47.5) × (10–) 14.5–18.5(–20) µm, length/width ratio 1.8–3, average 2.4 (dry material) or 2.1–3.4, average 2.5 (fresh), wrinkling pattern of outer wall angular, of end wall fibrillar; germ tubes subapically inserted, non- to two-septate, short to long, 20–115 × 2.5–6.0 µm, germ tubes terminating simply or in a club-shaped or rarely in a lobed appressorium, sometimes branching.

Erysiphe cf. ***macleayae*** R.Y. Zheng & G.Q. Chen on *Chelidonium majus* PLATE 2

SPECIMENS STUDIED — On *Chelidonium majus* L.: Germany, Brandenburg, Potsdam, Lennéstr./Carl-von-Ossietzky-Str., 4 Oct. 2004, leg. V. Kummer (KR 0021909 and

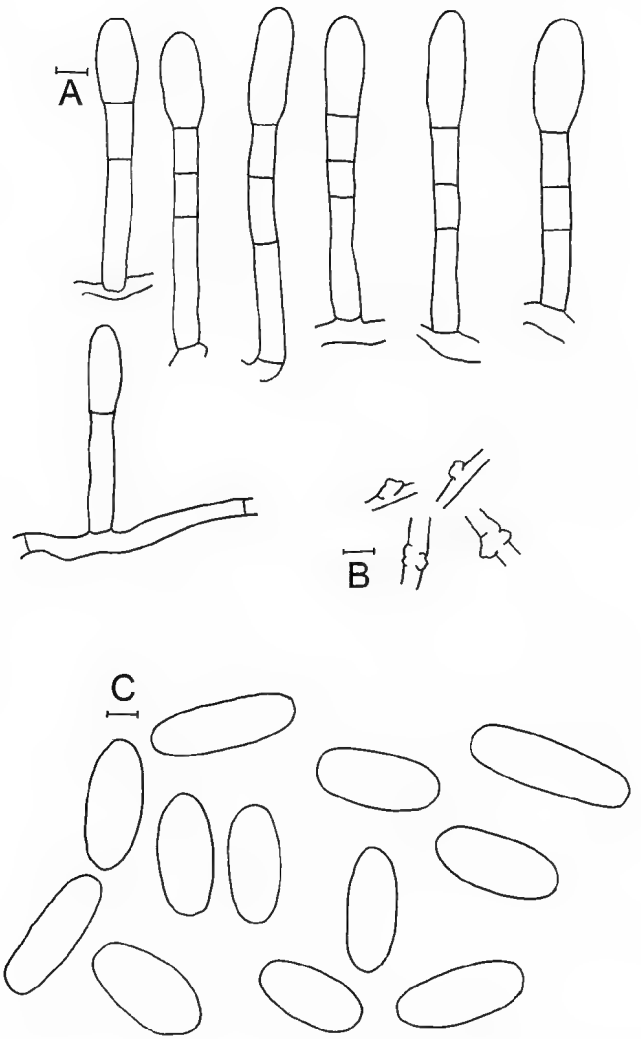

Plate 1. *Erysiphe macleayae* on *Macleaya* cf. *cordata* (KR 0021921 KM 203). A. Conidiophores (epiphyllous), B. Appressoria, C. Conidia. Bar 10 µm; drawings by A. Schmidt.

herbarium A. Schmidt no. KM 202) (fresh); Brandenburg, Potsdam, Maulbeerallee 2, 25 Sep. 2005, leg. V. Kummer (KR 0021933 and herb. A. Schmidt no. KM 212) (fresh); Thüringen, Jena, near University, Institut für Ökologie, near Dornburger Straße, 18 Oct. 2005, leg. V. Kummer (KR 0021934 and herbarium A. Schmidt no. KM 215); Thüringen, Bad Frankenhausen, on town wall between Fritz-Brather-Str. and Franz-Winter-Str., 14 Oct. 2007, leg. A. Schmidt (KR 0002945 and herbarium A. Schmidt no. KM 250) (fresh); Baden-Württemberg, Karlsruhe, Grünwinkel, Albsiedlung, Silcherstr., slope, 110 m, 13

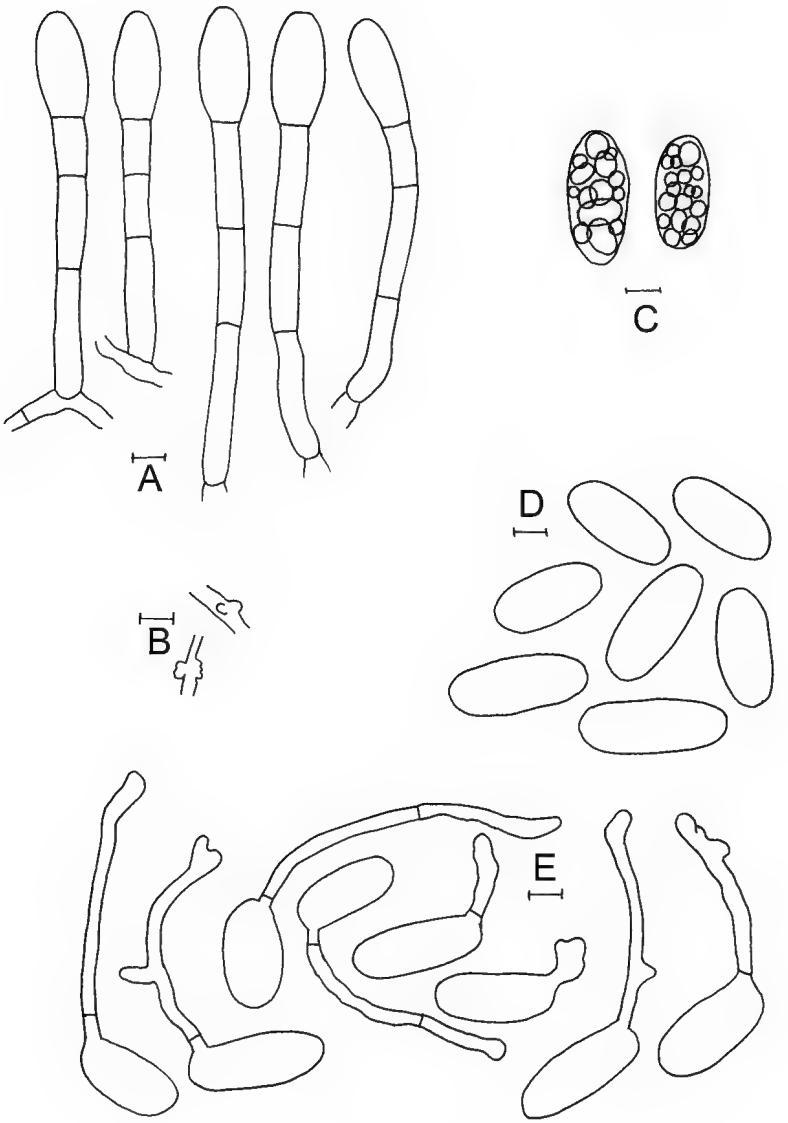

Plate 2. *Erysiphe* cf. *macleayae* on *Chelidonium majus*. A. Conidiophores (epiphyllous) (KR 0021909 = KM 202), B. Appressoria (KR 0021934 = KM 215), C. Conidia with oil drops (KR 0004850), D. Conidia (KR 0021909 = KM 202), E. Germinated conidia after 24 h in moist chamber cultures (KR 0021909 = KM 202). Bar = 10 µm; drawings 2A, B, D, E by A. Schmidt, 2C by M. Scholler.

Sep. 2009, leg. M. Scholler (KR 0004850) (fresh). Hungary, Martonvásár, 9 Sep. 2004, leg. et det. T. Jankovics (2007) as «*Oidium* sp. » (HAL 1842 F) (dry).

Mycelium not conspicuous, not dense, arachnoid, whitish, amphigenous, predominantly epiphyllous, often around leaf veins and leaf margins; sterile hyphae superficial, hyaline, sparingly branched, smooth, 3.5–6 µm wide; hyphal appressoria rarely formed, lobed or nipple-shaped, single or sometimes in pairs; epiphyllous conidiophores 60–115 µm, 7–10.4 µm wide, sometimes slightly attenuated toward base, with uppermost cell widest, 3-celled, rarely 2-celled, foot-cell, longest cell of conidiophore, 31–70 µm, often curved, uppermost cell often shortest; conidia formed singly, ellipsoid, partly cylindrical, (28–) 31–40(–46.5) × (11.5–)12.5–17.5(–18.5) µm, length/width ratio 2.1–3 (average 2.4), wrinkling pattern of outer wall with angular ridges, partly without ridges; germ tubes subapically inserted, non- to two-septate, first septum close to conidium, germ tubes short to long, 15–125 × 3.5–6.0 µm, terminating simply or in a club-shaped or, if in short germ tubes, then in a lobed appressorium, sometimes branching.

Erysiphe cruciferarum Opiz ex L. Junell PLATE 3

SPECIMENS STUDIED — On *Eschscholzia californica* Cham.: Germany, Niedersachsen, Soltau, Heidepark, wayside (MTB 2925/3), 57 m, 23 Sep. 2009, leg. J. Kruse (herbarium J. Kruse no. E 0279, mixed infection with *Acroconidiella eschscholziae* (Harkn.) M.B. Ellis) (dry).

Mycelium not conspicuous, sparse, whitish, amphigenous, mainly hypophyllous and caulicolous; sterile hyphae superficial, hyaline, sparingly branched,

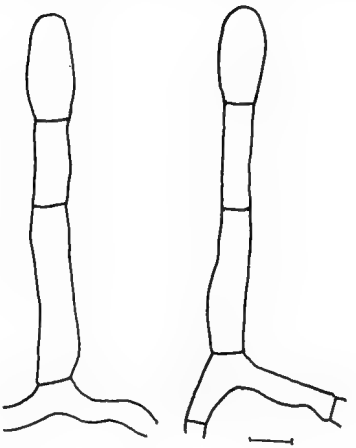

PLATE 3. *Erysiphe cruciferarum* on *Eschscholzia californica* (herb. J. Kruse no. E 0279). Conidiophores (hypophyllous). Bar = 10 µm; drawings by M. Scholler.

smooth, 4–10 µm wide; hyphal appressoria lobed; hypophyllous conidiophores central on mother cell, 55–70(–100) µm, 7.5–9.5 µm wide, sometimes slightly attenuated, 2–3(–4)-celled, foot-cell, longest cell of conidiophore, straight or rarely slightly curved; conidia formed singly, cylindrical to ellipsoid, 37.5–52.5 × (12.5–)14.5–18.5 µm, length/width ratio 2–3.9 (average 2.9), wrinkled wall surface angular; germ tubes inserted subapically, non- to one-septate, 20–55 × 3.5–5.5 µm, terminating simply or in a club-shaped appressorium.

Oidium sp.

Specimens studied On *Pseudofumaria lutea* (L.) Borkh. (≡ *Corydalis lutea* (L.) DC.): Germany, Brandenburg, Potsdam-Bornim, Florastr. 4, 2 Oct. 2004, leg. et det. V. Kummer as «cf. *E. werneri*» (HAL 2123 F) (dry); Germany, Sachsen-Anhalt, Tangermünde, "Roßfurth", Elbtor, shady wall, 8 Oct. 2004, leg. et det. H. Jage as «*Oidium*» (HAL 2124 F) (dry, scanty material).

Mycelium conspicuous, dense, whitish, amphigenous, predominantly hypophyllous; sterile hyphae hyaline, branched, smooth, 4–6.5 µm wide; hyphal appressoria lobed or nipple-shaped in pairs; conidiophores 65–130 µm long; epiphyllous 65–75 µm, hypophyllous 95–125 µm, uniformly 5.5–9 µm wide, not attenuated toward base, (2–)3(–4)-celled, foot-cell, longest cell of conidiophore, straight or sinuous; conidia formed singly, cylindrical to ellipsoid, 29.5–50.0 × 9–12(–13) µm, length/width ratio 2.3–5.3 (average 3.6), wrinkled outer wall angular; germ tube subapically inserted, non-septate, 20.5 × 3 µm, terminating simply.

Species on *Hydrangeaceae*

Oidium hortensiae Jørst. Plate 4

Specimens studied On *Hydrangea macrophylla* (Thunb.) Ser.: Germany, Berlin, Lichterfelde, Hortensienstr., balcony, flower-pot, 27 Jun. 1993, leg. M. Scholler (KR 0003726) (fresh); Hamburg-Nienstedten, Cordsstr. 3, Cords nursery, flower-pot, 26 Jun. 2009, leg. A. Schmidt (KR 0024164, herbarium A. Schmidt no. KM 275) (fresh).

Mycelium not conspicuous, not dense, arachnoid, whitish, in small patches on violet spots, amphigenous; hyphal appressoria numerous, lobed, in pairs, often in sequences of several pairs (up to 4); epiphyllous and hypophyllous conidiophores about central on mother cell, 40–130 µm long, 7–10 µm wide, mostly 2–3-celled, rarely 4–5-celled, foot-cell 17–45(–50) µm long, mostly straight, sometimes attenuated toward base or curved, 4–5-celled conidiophores occur on both sides of the leaf, conidiophores longer and slightly narrower below; conidia formed singly, ellipsoid to cylindrical, partly doliiform or ovoid, (25–)27–40(–45) × (11.5)–13–22 µm, length/width ratio 1.7–3.1 (average 2.2), wrinkling pattern of outer wall with mainly longitudinal, slightly angular ridges; germ tubes subapically inserted, one-septate, 20–88 × 2–5 µm, germ

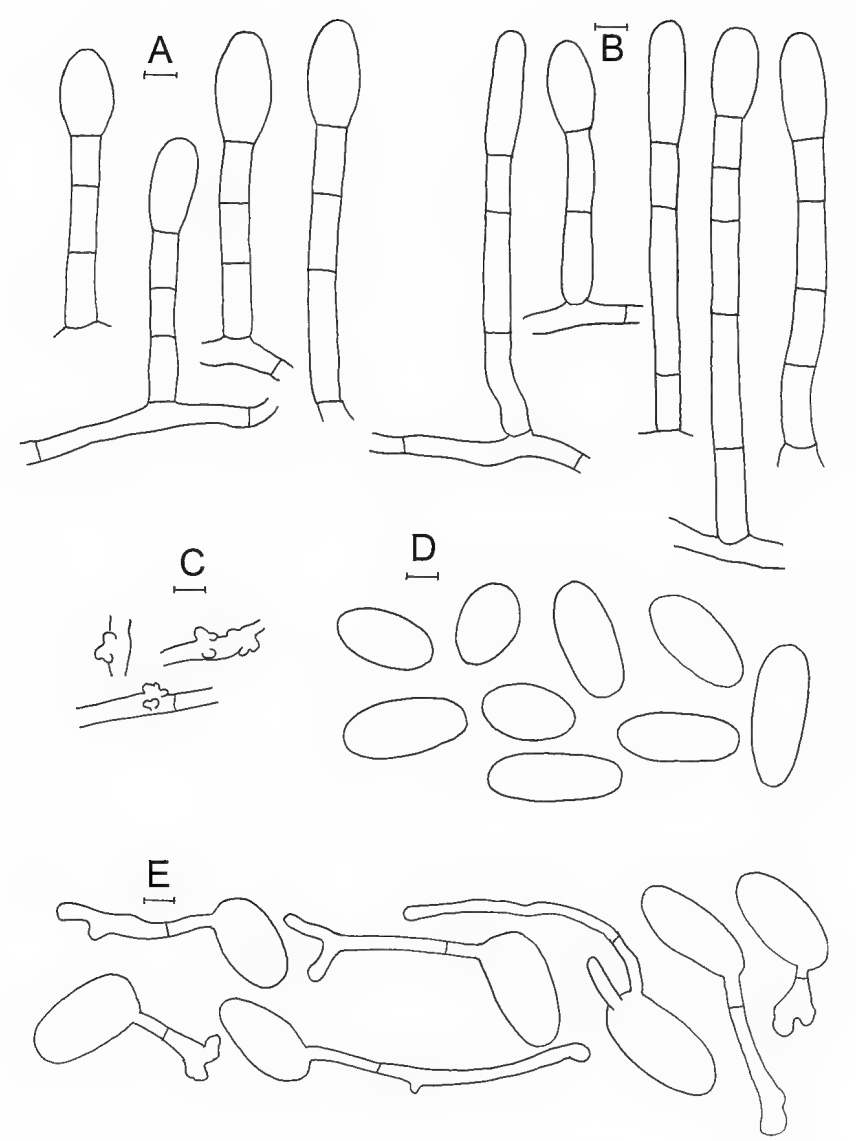

PLATE 4. *Oidium hortensiae* on *Hydrangea macrophylla* (KR 0024164 = KM 275). A. Conidiophores (epiphyllous), B. Conidiophores (hypophyllous), C. Appressoria, D. Conidia, E. Germinated conidia after 24 h in moist chamber cultures. Bar = 10 µm; drawings by A. Schmidt.

tubes short to long, rarely terminating simply, mostly in a club-shaped or lobed appressorium, sometimes branched, often germinating with two or three germ tubes per conidium.

Erysiphe deutziae (Bunkina) U. Braun & S. Takam. on *Deutzia* PLATE 5

SPECIMENS STUDIED — On *Deutzia* cf. *scabra* Thunb.: Germany, Hamburg-Nienstedten, Wesselhoeftpark, 27 Jun. 2009, leg. A. Schmidt (KR 0024165, herbarium A. Schmidt no. KM 276/1) (fresh); Hamburg-Nienstedten, Wesselhoeftpark, 18 Jul. 2009, leg. A. Schmidt (KR 0024168, herbarium A. Schmidt no. KM 276/2) (fresh).

Mycelium not conspicuous, not dense, whitish, epiphyllous, low rate of infection; hyphal appressoria numerous, lobed, in pairs; epiphyllous conidiophores 55–65 µm long, 8–10 µm wide, sometimes slightly attenuated toward base, 2–3-celled, foot-cell 25–35 µm long, straight or sometimes curved; conidia formed singly, mostly ellipsoid, partly ovoid or doliiform, 26.5–36(–40) × (16.5–) 18–20 µm, length/width ratio 1.4–2.2 (average 1.7), fresh conidia with numerous small conspicuous droplets beside large oil drops, wrinkling pattern of outer cell wall irregular with interrupted longitudinal and angular ridges; germ tubes subapically inserted, always one-septate, septum distant from conidium, mostly about middle of germ tube, germ tubes short to moderately long, 25–125 × 3.0–4.5 µm, terminating in a mostly lobed or club-shaped appressorium.

Erysiphe deutziae (Bunkina) U. Braun & S. Takam. on *Philadelphus* PLATES 6–8

SPECIMENS STUDIED — On *Philadelphus* cf. *coronarius* L. (cult.): Germany, Nordrhein-Westfalen, University campus, 20 Mar. 2003, leg. G. Feige & N. Ale-Agha (published as *Oidium* cf. *hortensiae*, see Ale-Agha et al. 2008), Baden-Württemberg, Karlsruhe-Grünwinkel, Silcherstr. 25, garden, 20 Aug. 2008, leg. M. Scholler (KR 0025921, herbarium A. Schmidt no. KM 269/1) (fresh); Baden-Württemberg, Karlsruhe-Grünwinkel, Silcherstr. 25, garden, 26 Aug. 2008, leg. M. Scholler (KR 0025922, herbarium A. Schmidt no. KM 269/2) (fresh).

Mycelium not conspicuous, not dense, whitish, arachnoid, amphigenous and on fruits; hyphal appressoria numerous, lobed, mostly in pairs; epiphyllous conidiophores 50–75 µm long, 8.5–12.0 µm wide, slightly attenuated toward base, 2–3-celled, upper cell sometimes slightly swollen, foot-cell 21–31 µm, straight or sometimes slightly curved, hypophyllous conidiophores not different in length; conidia formed singly, mostly ellipsoid, partly ovoid or doliiform, 24–34.5 × (16.5–)17.5–20(–22) µm, length/width ration 1.3–1.9 (average 1.6), fresh conidia with numerous small conspicuous oil drops beside large oil drops, wrinkling pattern of outer cell wall with mainly longitudinal ridges; germ tubes subapically inserted, non- to one-septate, septum distant from conidium, about middle of germ tube, germ tubes short to long, 20–84 × 3.5–5.5 µm, terminating simply, in a lobed or club-shaped appressorium.

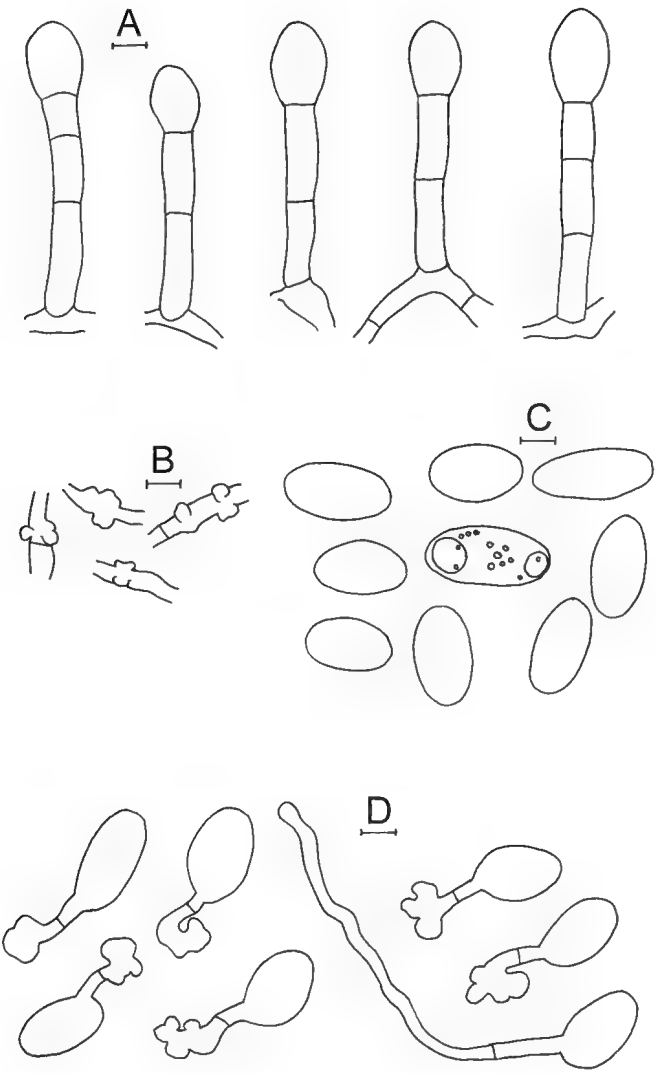

PLATE 5. *Erysiphe deutziae* on *Deutzia* cf. *scabra* (KR 0024165 KM 276/1). A. Conidiophores (epiphyllous), B. Appressoria, C. Conidia, D. Germinated conidia after 24 h in moist chamber cultures. Bar 10 µm; drawings by A. Schmidt.

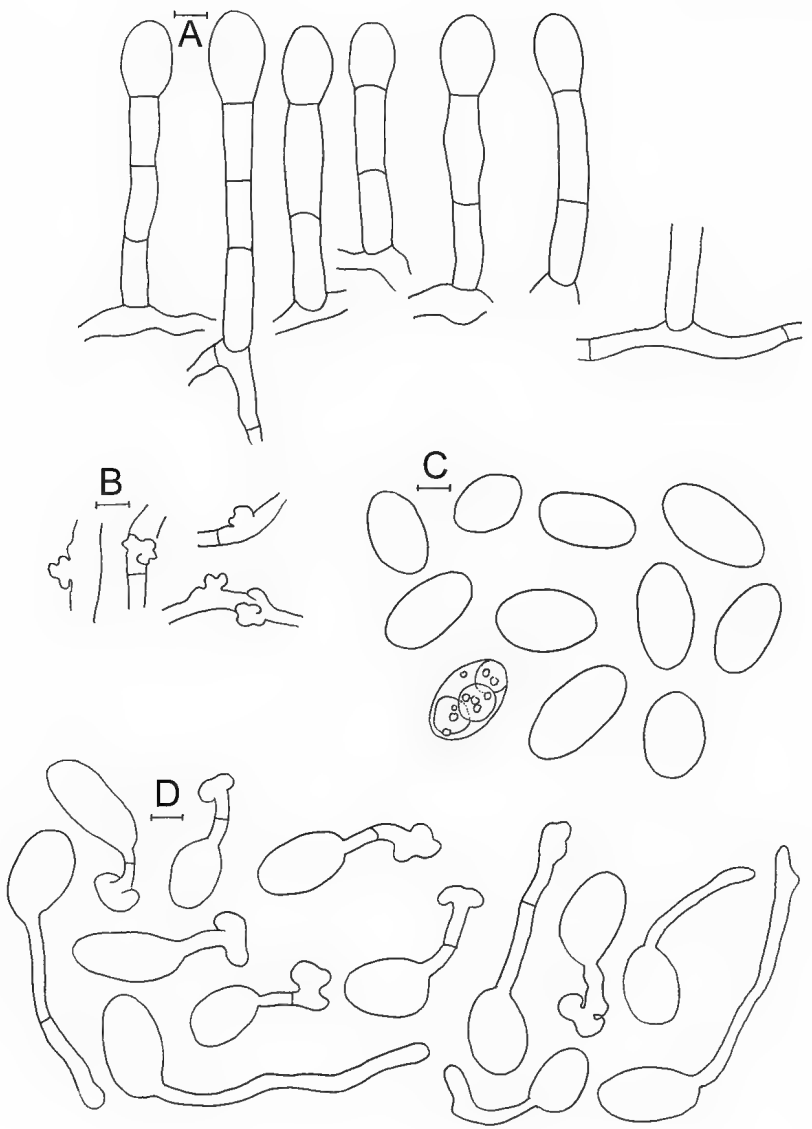

Plate 6. *Erysiphe deutziae* on *Philadelphus* cf. *coronarius*. A. Conidiophores (epiphyllous) (KR 0025921 = KM 269/1), B. Appressoria (KR 0025921 = KM 269/1), C. Conidia (KR 0025921 = KM 269/1), D. Germinated conidia after 24 h in moist chamber cultures (KR 0025922 = KM 269/2). Bar = 10 µm; drawings by A. Schmidt.

PLATE 7. Germinating conidia of *Erysiphe deutziae* on *Philadelphus* cf. *coronarius* (KR 0025922 = KM 269/1). Bar = 10 µm; photograph by A. Schmidt.

PLATE 8. Anamorph of *Erysiphe deutziae* on fruits of *Philadelphus* cf. *coronarius* in a garden in Karlsruhe. 24 Aug. 2008; photograph by M. Scholler.

Key to anamorphs of powdery mildew species recorded or expected in Germany

On *Papaveraceae* (incl. *Fumariaceae*)

1a. Conidia formed in chains; on *Papaver rhoeas* ..... ***Golovinomyces orontii***

1b. Conidia formed singly 2

2a. Mycelium internal and external; conidiophores mostly emerging through stomata; conidia distinctly dimorphic, primary conidia lanceolate, apically attenuated, secondary conidia ellipsoid-cylindrical; on *Glaucium* spp., *Papaver* spp. ..... ***Leveillula taurica***

2b. Mycelium external; conidiophores arising from superficial hyphae; conidia + ellipsoidal 3

3a. Conidiophore foot-cells usually straight, rarely sinuous; on *Eschscholzia californica*, ?*Fumaria officinalis*, *Glaucium flavum*, *Papaver argemone*, *P. dubium*, *P. nudicaule*, *P. orientale*, *P. rhoeas*, *P. somniferum* ..... ***Erysiphe cruciferarum***

3b. Foot-cells often sinuous, on other hosts 4

4a. Mycelium predominantly hypophyllous, average length/width ratio of conidia about 3.5; on *Pseudofumaria lutea* ..... ***Oidium*** sp.

4b. Mycelium predominantly epiphyllous, average length/width ratio of conidia below 3.5 (2 to 3); on other hosts 5

5a. Conidiophores mostly 60–115 µm long, often attenuated toward base; on *Chelidonium majus* ..... ***Erysiphe*** cf. ***macleayae***

5b. Conidiophores shorter, mostly 50–85 µm, often attenuated toward base; on *Macleaya cordata, Meconopsis cambrica* ..... ***Erysiphe macleayae***

On *Hydrangeaceae*

1a. Conidia formed in chains; on *Hydrangea* spp. ..... ***Golovinomyces orontii***

1b. Conidia formed singly 2

2a. Conidiophores 2–3-celled, rarely 4–5-celled, conidia without small oil drops, conidia often germinating with two or three germ tubes per conidium; on *Hydrangea macrophylla* ..... ***Oidium hortensiae***

2b. Conidiophores only 2–3-celled, conidia with numerous small oil drops, generally germinating with only one germ tube per conidium; on *Deutzia* ×*magnifica*, *D. scabra* and *Philadelphus coronarius* ..... ***Erysiphe deutziae***

Discussion

Species on *Papaveraceae*

Erysiphe macleayae is an introduced species native of Asia (Braun 1987) that was previously reported from Germany by Ale-Agha et al. (2008) on *Macleaya cordata*. The anamorph of this species is reported here from Germany on *Macleaya* (HAL 2133 F, KR 0021921) and *Meconopsis* .The features of the anamorph and only ascomata on *Meconopsis* (herb. J. Kruse no. E 0293) agree

with the short description of *E. macleayae* on *Macleaya cordata* by Braun (1987). The specimen HAL 2209 F on *Macleaya* is heavily infested by the hyperparasite *Ampelomyces quisqualis* Ces., which obscures the morphological characters of the powdery mildew. HAL 2133 F contains many germinated conidia. *Meconopsis cambrica* is a new host genus and species for *E. macleayae*. *Erysiphe cruciferarum* has previously been listed on this host (Braun 1987: 204) but the specimens may belong to *E. macleayae*. A powdery mildew reported from Bulgaria on *Papaver somniferum* L. was identified as *E. macleayae* (Fakirova 1991) but U. Braun studied this material and determined that it belongs to *E. cruciferarum* (Braun 1995).

A powdery mildew on *Chelidonium majus* was first recorded in Italy by Ciferri & Camera (1962). This material is not available, so we do not know whether it is conspecific with the fungus on this host in Central Europe. The *Chelidonium* powdery mildew may be related to or even conspecific with *E. macleayae*. Minor differences are: number of septa in the conidial germ tubes, one versus up to two on *Chelidonium*; and conidiophore foot-cells up to 40 µm versus up to 70 µm on *Chelidonium*.

Jage et al. (2010) identified specimens HAL 2123 F and HAL 2124 F on *Pseudofumaria lutea* as *E. cruciferarum*. We re-studied this material and it differs from the anamorph of *E. cruciferarum* by: 1) often sinuous foot cells, 2) an extraordinarily high length/width ratio – 3.6, and 3) formation of predominantly hypophyllous mycelium. Braun (1987) reports a "larger than 2" l:w ratio for *E. cruciferarum* and in the specimens we studied this averaged between 2.4 and 2.9. Braun (1987) also reported that in *E. cruciferarum* the mycelium is formed amphigenously. Therefore, it is doubtful that the *Pseudofumaria* powdery mildew is conspecific with *E. cruciferarum*.

One other species, *E. werneri* U. Braun, occurs on *Papaveraceae* (*Corydalis* s. str.). The anamorph of this species bears some resemblance to the oidial state on *Pseudofumaria*. Based on the protologue, *E. werneri* forms large conidia (48–63 × 15–23 µm). In a recent description of *E. werneri* from Korea, Shin (2000) reported conidia 35–48 × 13–18(–20) µm with single hyphal appressoria and mainly two-celled conidiophores. This agrees with the German material except that it is not as wide, 9–12 (–13) µm, has hyphal appressoria, and mainly three-celled conidiophores.

Species on Hydrangeaceae

The anamorph *Oidium hortensiae* occurs on *Hydrangea* (Blumer 1967; Braun 1987, 1995). Described as straight, cylindrical, we determined that the conidiophores may also be attenuated toward base or curved. In contrast to Braun (1987, 1995), we did not find conidiophores producing conidia rarely in short chains. Our germination experiments indicated that germ tubes mostly produced a club-shaped or lobed terminal appressorium. Simple germ tubes

without any appressoria were rare. In this respect, our observations agree with those of Blumer (1967) but disagree with Braun (1987) who stated that the species was "without terminal lobed appressorium". According to Braun (1995), *Hydrangea macrophylla* may be infected by another powdery mildew, *Golovinomyces orontii*. This species is known from Switzerland and may be expected in Germany.

A detailed description of the anamorph of the Asian *Erysiphe deutziae* on *Deutzia* spp. in Europe was provided by Bolay et al. (2005). Our observations agree with their description and provide additional information on the mode of germination and germ tube morphology. However, Bolay et al. (2005) described the conidia as 12.5–15 µm wide while the German specimens were (16.5–)17.5–20(–22) µm wide. This difference may be because Bolay et al. (2005) measured dried stained conidia whereas we measured only fresh conidia. In his monograph, Braun (1987) described them as 14–20 µm wide. An important diagnostic feature of *E. deutziae* is the formation of numerous small refractive droplets in the conidia; these are clearly visible in fresh conidia. The smaller droplets are formed in addition to larger oil drops that are common in most powdery mildews. Interestingly, in contrast to the large drops, the small drops are visible even in dried conidia (when rehydrated in water). The anamorph on *Philadelphus* agrees with *E. deutziae* on *Deutzia* and we therefore consider them to belong to the same species. This also applies to HAL 2138 F, which was previously published as *Oidium* cf. *hortensiae* (Ale-Agha et al. 2008). The typical *Oidium hortensiae* differs from *E. deutziae* by several morphological characters, e.g. by not forming small droplets. Also infection experiments reported by Blumer (1928, cited after Blumer 1933) support our results. This study reports that *Oidium hortensiae* infects various *Hydrangea* species but not *Deutzia* and *Philadelphus* species.

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Paecilomyces wawuensis, a new species isolated from soil in China

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ABSTRACT — A new species, *Paecilomyces wawuensis*, isolated from soil samples of the Wawu Mountain, Sichuan Province, China, is described and illustrated. It is characterized by phylogenetic analysis of ITS rDNA and β-tubulin gene sequences, white obverse and sandy beige reverse colonies that grow slowly on Czapek agar, cylindrical phialides with long, thin necks, and ellipsoidal-ovate, spinulose conidia.

KEY WORDS — taxonomy, fungi, hyphomycete

Introduction

The hyphomycete genus *Paecilomyces* was introduced by Bainier (1907) to accommodate *Paecilomyces variotii* Bainier, a species growing in the air, soil, and compost and on wood. It was characterized by verticillate conidiophores with divergent whorls of phialides, which have a cylindrical or inflated base tapering into a long and distinct neck. The conidia are typically hyaline, one-celled, smooth-walled, and produced in basipetal chains. The genus was revised by Brown & Smith (1957) and further modified by Samson (1974), who accepted 31 species. Some species of *Paecilomyces* were later found to represent anamorphs of *Byssochlamys* Westling, *Talaromyces* C.R. Benj., *Thermoascus* Miehe, *Cordyceps* Fr., and *Torrubiella* Boud. (Stolk & Samson 1972, Samson 1974).

In *Paecilomyces,* most species can be separated from each other with colonies, conidiogenous cells, conidia, and phialides. Han (2007) accepted only 64 of the 125 published *Paecilomyces* species. According to Index Fungorum (www.indexfungorum.org), over 140 epithets were assigned to *Paecilomyces;* many of them, however, are known to be taxonomic synonyms or dubious taxa.

A total of 39 species in *Paecilomyces* have been reported in China, including 18 entomogenous species and 21 species from soil (Liang et al. 2005a,b, 2006; Han et al. 2005a,b,c; Li et al. 2006).

Based on the records of *Paecilomyces* and the distinctive molecular and morphological characteristics, the fungus isolated from soil samples of the Wawu Mountain, Sichuan Province, China, is described as a new species of *Paecilomyces*.

Materials & methods

Collection and strain isolation

Strain GZU-BCECWS15 was isolated from a soil sample collected from the Wawu Mountain, Sichuan Province, China, in July, 2008. Two grams of soil were added to a flask containing 20 ml sterilized water and glass beads. The soil suspension was diluted to a concentration of 10^{-1}–10^{-3} after shaking for about 10 min. A 1 ml soil suspension (concentration 10^{-3}) was mixed with Martin medium in sterilized 9 cm diam Petri dish and incubated at 25°C for 5 days. The strain was purified by transplanting to Martin's slants.

Morphological identification of strain

The strain studied was transplanted onto Czapek agar, potato dextrose agar (PDA), and Sabouraud agar. After incubating at 25°C for 14 days, morphological identification of the strain was carried out based on colony characters, conidiogenous structure and other biological features (Brown & Smith 1957, Samson 1974).

The generic names *Paecilomyces* and *Penicillium* are abbreviated as "*P.*" and "*Pen.*" respectively. The holotype GZU-BCECWS15 of *P. wawuensis* was deposited in the Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, PR China.

Table 1. ITS rDNA sequences from GenBank used in the phylogenetic analysis

Name	GenBank No.	Name	GenBank No.
P. antarcticus	AJ879113	*P. gunnii*	AJ309339
P. carneus	AY624171	*P. marquandii*	AY624193
P. carneus	AB103179	*P. niphetodes*	AY624192
P. carneus	DQ888728	*P. penicillatus*	AY624194
P. carneus	DQ914684	*P. viridis*	AY624197
P. gunnii	AJ309343	*P. wawuensis*	GU453921
P. gunnii	GY453920	*Pen. expansum*	AF404655

Table 2. β-tubulin gene sequences from GenBank used in the phylogenetic analysis

Name	GenBank No.	Name	GenBank No.
P. carneus	AY624209	*P. viridis*	AY624235
P. carneus	AY624210	*P. wawuensis*	HM480496
P. gunnii	HM480497	*Pen. expansum*	AF003248

DNA sequencing & phylogenetic analysis

Genomic DNA was extracted from fungal mycelia directly collected from the plates following the CTAB isolation protocol of Oscar et al. (1999). Template DNA (30–40 ng) was amplified in a 50 µl PCR reaction mixture consisting of 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8), 6 mM $MgCl_2$, and 500 µM each of dATP, dCTP, dGTP, and dTTP, with 60 pmols ITS4 & ITS5 primers (White et al. 1990) for ITS rDNA or T1&T22 primers (O'Donnell & Cigelnik 1997) for β-tubulin gene, and 2 units TaqDNA polymerase (TianGen, China). The reaction was set up as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min in Gene Amp PCR system 9700 (Gene Amp, U S). The temperature of annealing for β-tubulin gene (TUB2) was 54°C.

The PCR products were purified by using a UNIQ-10 PCR Purification Kit (Sangon Biotech, China). The purified PCR products were sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut) with ITS4 or ITS5 and T1 or T22. The DNA sequences of ITS rDNA and β-tubulin gene of the fungus have been submitted to GenBank.

Nucleotide sequences of ITS rDNA and β-tubulin gene of the isolate GZU-BCECWS15 and their allies as well as the outgroups retrieved from GenBank were assembled using Tex-Edit Plus (Bender, TomBB@aol.com) respectively. The alignment of the sequence files was conducted using the CLUSTAL W software (Thompson et al. 1994). Phylogenetic analyses were performed with PAUP version 4.0b10 (Swofford 2004). The most parsimonious trees (MPT) were determined from the data sets using the heuristic search options with 1000 random sequence input orders with MULPARS on and TBR branch swapping for the exact solution. The unconstrained topologies of the equally parsimonious trees were compared using the Kishino-Hasegawa test of PAUP. The best topology was selected as the most parsimonious tree topology. Parsimony bootstrap with 1000 replicates in PAUP was applied to the tree to evaluate the stability. Other measures including tree length, consistency, retention, rescaled consistency and homoplasy indexes (TL, CI, RI, RC and HI) were also calculated.

Taxonomy

Paecilomyces wawuensis Jin He, J.C. Kang & B.X. Lei, **sp. nov.** FIG. 1

MycoBank MB 518791

On agaro Czapekii, coloniae 25–26 mm diam in 14 diebus ad 25°C, albae, floccosae, margine regularis, reversum vinacea. Hyphae hyalinae, septatis, levibus, 1.3–1.5 µm crassis. Conidiophora breva, simplicia, 16–40 × 1.3–2.0 µm, phialides singulare vel phialidibus 2 ad 4 terminatis, Phialides12–42 × 1.8–4.0 µm, cylindricae, e basi inflata, angustatae collolongo minus quam 0.4 µm. Conidia monocellula, hyalina, verrucosa, ellipsoidea ad ovata vel subglobosa, 4.3–6.9 × 3.3–5.3 µm, facientia divergentes, catenas exsiccates.

Typus: *GZU-BCECWS15 et cultara , isolatus ab soli in solo Wawu, Provinice Sichuan, VII.2008, T.C. Wen; in Guizhou Univ., conservatur.*

Colonies on Czapek agar growing slowly, attaining a diameter of 25–26 mm 14 days at 25°C; white, ridged, dense floccose, thick, with orderly margins, with some irregular radiating furrow and more or less hyaline exudates; reverse

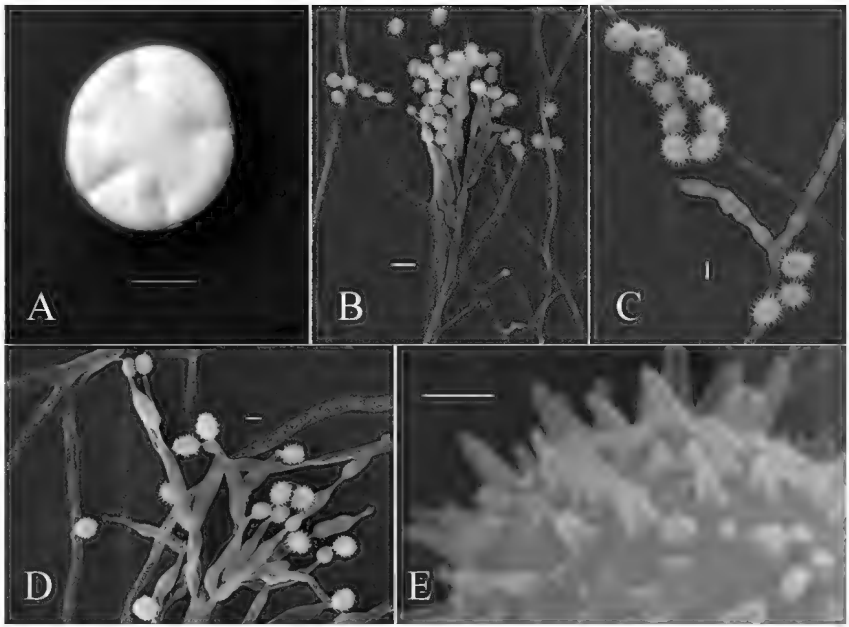

FIG. 1. Photographs of colony and conidiogenous structure of *Paecilomyces wawuensis* under scanning electron microscope. **A.** Colony on Czapek agar after 14 days at 25°C. **B.** Conidiogenous structure. **C.** Ellipsoidal conidia in chains. **D.** Phialides of the conidiogenous structure. **E.** Echinula of a conidium. Scale bars: **A** 10 mm, **B** 5 μm, **C–D** 2 μm, **E** 0.5 μm.

sandy beige, appearing soluble wine red pigment. Vegetative hyphae septate, hyaline, smooth-walled, 1.3–1.5 μm wide. Conidiophores mononematous erect or absent, hyaline, smooth-walled, uneven length, 16–40 × 1.3–2.0 μm, with single phialide or whorls of 2 to 4 phialides, or phialides growing from hyphae directly. Phialides 12–42 × 1.8–4.0 μm, consisting of a cylindrical, somewhat inflated base, tapering into a long and thin neck, somewhat incurved, 6.0–6.7 μm long and less than 0.6 μm wide. Conidia one-celled, hyaline, roughened-walled with spinulose, most ellipsoidal or ovate to subglobose, 4.3–6.9 × 3.3–5.3 μm, forming divergent, dry and basipetal chains. Chlamydospores present, produced singly or in short chains, thick-walled, roughened, globose to subglobose, 7.5–8.5 μm in diameter.

COLONIES ON PDA at 25°C within 14 days, usually growing more rapidly than on Czapek agar, 27mm, consisting of a pink mycelium, with some irregular radiating furrow and more or less hyaline exudates. Reverse saddle brown, with distinct radiating furrow and present wine red pigment diffusion.

COLONIES ON SABOURAUD AGAR 25 mm at 25°C within 14 days, consisting of white mycelium, with some irregular radiating furrow. Reverse light brown, with distinct radiating furrow.

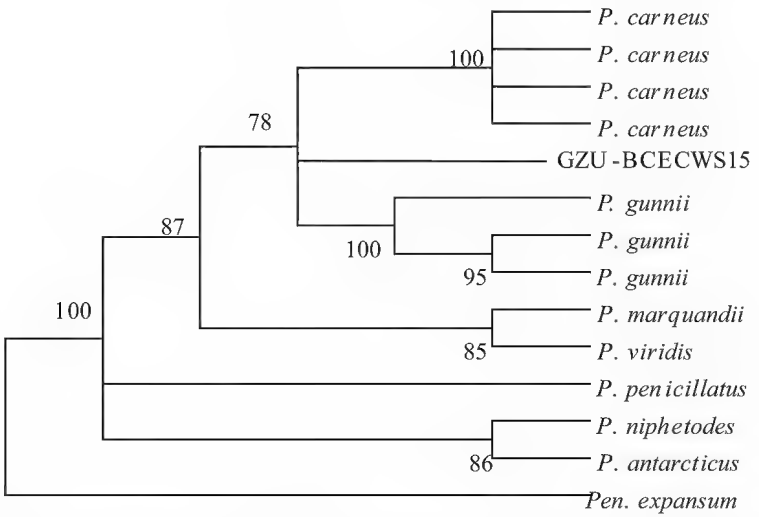

FIG. 2. The gene tree was constructed using the MP method and based on phylogenetic analysis of the nucleotide sequences of ITS1-5.8S-ITS2 rDNA. It shows the relationships between GZU-BCECWS15 and other *Paecilomyces* species. Bootstraps values (1,000 replicates) are indicated at the nodes (TL 577, CI 0.825, HI 0.175, RI 0.710, RC 0.586).

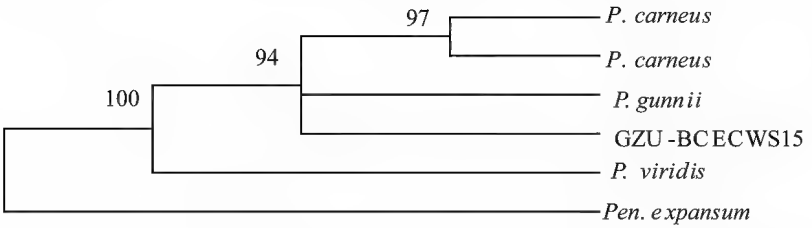

FIG. 3. The gene tree was constructed using the MP method and based on phylogenetic analysis of the β-tubulin gene sequences. It shows the relationships between GZU-BCECWS15 and other *Paecilomyces* species. Bootstraps values (1,000 replicates) are indicated at the nodes (TL 174, CI 0.937, HI 0.063, RI 0.645, RC 0.604).

Phylogenetic analyses

The ITS1-5.8S-ITS2 rDNA of the *P. wawuensis* has been sequenced and compared with that of *P. carneus* (CBS 239.32, GenBank Accession No.: AY624171) and *P. gunnii* (GenBank Accession No.: AJ3093390) in an alignment. In comparison with *P. carneus*, *P. wawuensis* exhibited 31 transitions/transversions and 36 deletion and 6 insertion substitutions. Meanwhile there were 60 positional transitions/transversions and 57 insertion/deletion substitutions in ITS1-5.8S-ITS2 rDNA between *P. gunnii* and *P. wawuensis*.

At the same time, the alignment of β-tubulin gene sequences from *P. gunnii, P. carneus,* and *P. wawuensis* showed that *P. wawuensis* had 1 insertion substitution, 6 deletion substitutions and 12 transitions/transversions both to *P. gunnii* and *P. carneus*.

Both the most parsimonious trees (MPT) inferred from the ITS rDNA (Fig. 2) and the β-tubulin gene sequences data (Fig. 3) showed that the strain GZU-BCECWS15 stands parallel to the others, although it is clustered with *P. gunnii* and *P. carneus* in a subclade.

Discussion

In the genus *Paecilomyces*, rare species have been found to have echinulate conidia. The previously accepted species with echinulate conidia were *P. carneus* Brown & Smith (Samson 1974), *P. gunnii* Z.Q. Liang (Liang 1985), and *P. curticatenatus* Z.Q. Liang & Y.F. Han (Han et al. 2007). Liang et al. (2009) recombined *P. curticatenatus* in a new genus, *Taifanglania*, characterised by solitary phialides; this character separates *P. curticatenatus* from *P. wawuensis*. The new species *P. wawuensis* can be separated from *P. carneus* and *P. gunnii* by the sandy beige reverse of the colony on Czapek agar and the large ellipsoidal-ovate conidia (Table 3). *Paecilomyces wawuensis* can be distinguished from the other species in having a white/ sandy beige colony on Czapek agar and echinulate conidia.

Table 3. A comparison between *Paecilomyces wawuensis* and its related species

Species	Colony		Phialides	Conidia	Chlamydospores
	obverse	reverse			
P. carneus	white	olive drab	cylindrical	subglobose to ellipsoidal 3 4 × 2 2.5 μm	absent
P. gunnii	grey	brown	cylindrical	subellipsoidal or subovate 1.6 4.8 × 1.2 3.5 μm	present
P. wawuensis	white	sandy beige	cylindrical/ ampulliform	ellipsoidal or ovate to subglobose 4.3 6.9 × 3.3 5.3 μm	present

Meanwhile the dominant substitutions and transitions/transversions in the alignments of ITS1-5.8S-ITS2 rDNA and β-tubulin gene sequences of *P. carneus*, *P. gunnii,* and *P. wawuensis* differentiate the new species from the others.

In conclusion, *Paecilomyces wawuensis* can be distinguished from the other species in the genus by the following morphological characters: 1) colonies on Czapek agar grow slowly and the obverse is white while the reverse is sandy beige; 2) phialides consist of a cylindrical, sometimes inflated base, tapering into a long, thin, and sometimes incurved neck; and 3) conidia are ellipsoidal or ovate to subglobose, large and roughened-walled with spinules.

Both phylogenetic trees (Figs. 2, 3) based on ITS rDNA and β-tubulin gene sequences show that the strain GZU-BCECWS15 stands parallel to *P. carneus*

and *P. gunnii* in a sub-clade, although all of them possess roughened-walled conidia with spinules. Both molecular and morphological evidence supports *P. wawuensis* (GZU-BCECWS15) as a new species.

Acknowledgements

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Mycosphaerella nyssicola revisited: a species distinct from *M. punctiformis*

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ABSTRACT *Nyssa* trees involved in a breeding program for ornamentals were found to be affected deleteriously by a leaf spot disease. The causative agent was identified as a species of *Mycosphaerella* that had been classified as morphologically indistinguishable from *M. punctiformis*. A subsequent taxonomic investigation using morphological data from new and existing herbarium collections, cultural data, and ITS region rDNA sequences suggested that this was a distinct species on *Nyssa* that represents the previously described *M. nyssicola*. The species is lectotypified and epitypified to promote nomenclatural and taxonomic stability.

KEY WORDS *Ascomycota*, *Asteromella nyssae*, *Dothideomycetes*, lectotype, *Phyllosticta nyssae*

Introduction

Nyssa (*Cornaceae*) is a small genus of trees disjunct between eastern Asia and eastern North America and Central America (Wen & Stuessy 1993). Four North American species (*N. aquatica* L., *N. biflora* Walter, *N. ogeche* W. Bartram ex Marshall, and *N. sylvatica* Marshall), collectively known as gums or tupelos, are found in swamps and alluvial soils as well as uplands. The black gum, *N. sylvatica*, is the most commonly cultivated species for ornamental and urban tree use due to its beautiful red fall color. The USDA-ARS National Arboretum began a *Nyssa* breeding program in the early 1990's directed at improving urban adaptability and transplantability for the nursery industry. Increased use of *Nyssa* has been tempered by a disfiguring leaf spot disease that results in reduced aesthetic appeal and premature defoliation (Dirr 1998). In 2006, the Arboretum refocused the *Nyssa* program towards developing resistance to this organism.

Recent attempts at identifying the causal organism failed or resulted in isolation of secondary pathogens (Dirr 1998, Charles Hodges pers. comm.). It was assumed to be a widely distributed species of *Mycosphaerella* Johanson (*Dothideomycetes*, *Ascomycota*) that has been classified as morphologically indistinguishable from and probably conspecific with *M. punctiformis* (Pers.) Starbäck, the type species of the genus (Aptroot 2006). The genus *Mycosphaerella* includes a vast diversity of species on multitudinous hosts, and it is successful in several ecological roles, including most importantly as a plant pathogen (Verkley et al. 2004, Crous 2009). Species of *Mycosphaerella* are in general host specific, but several species have broader host ranges (Crous 2009). A recent paper serving to stabilize the taxonomic application of *M. punctiformis* suggested that this species, in a strict sense, was specific to *Quercus* (Verkley et al. 2004).

Knowledge of the identity and host range of the leaf spot fungus from *Nyssa* is important in the development of disease control strategies and resistant cultivars. New isolates were obtained from *Nyssa* in field plots of the USDA breeding program and DNA sequence data of the ITS region were generated for comparison with other *Mycosphaerella* species. New specimens were compared with herbarium collections including type specimens, which are designated herein to promote nomenclatural and taxonomic stability.

Materials & methods

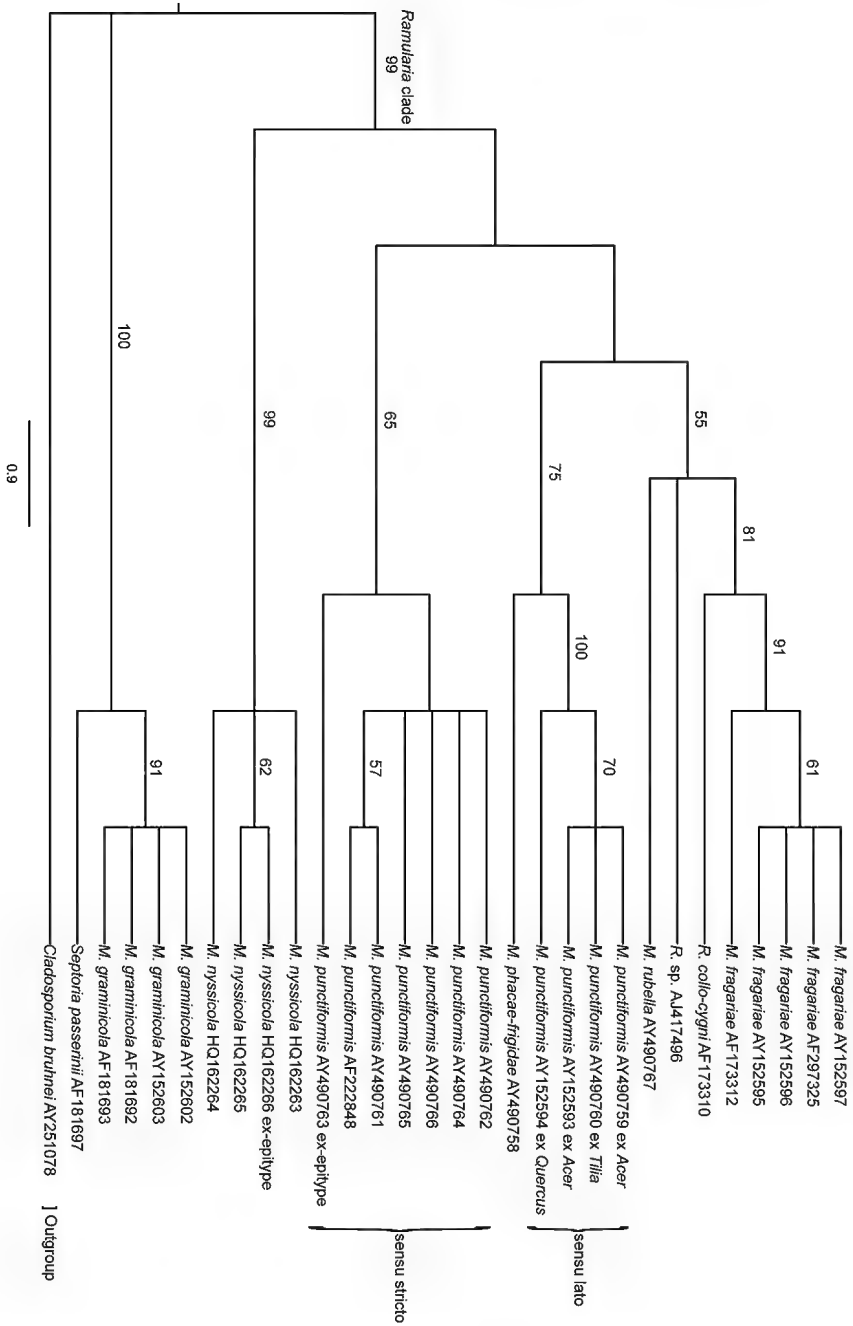
Isolation from fruiting bodies

Overwintered, dead, and fallen leaves were collected in spring. Cultures on cornmeal dextrose agar (CMD) were obtained variously by growth at room temperature from ascospores ejected from bits of leaves containing pseudothecia glued to the top lid of Petri dishes that were incubated with either the top lid oriented upwards or downwards or ascospores, usually with associated asci, from crushed pseudothecia isolated with a pipette. Cultures were deposited in the Centraalbureau voor Schimmelcultures (CBS).

Morphological examination

Microscopic observations and measurements of sections and crush mounts of fresh material were made in water. For viewing of dried material, material was wetted with 95% ethanol and subsequently rehydrated and viewed in 3% KOH. Phloxine solution (Largent et al. 1977) was also used to aid in microscopic examination. In the descriptions, an ^{m} indicates average or mean and Q is the length to width ratio. For phenotypic observations, cultures were grown on potato dextrose agar (PDA). See Farr & Rossman (2010) for complete collection data of examined historical herbarium collections. New collections were deposited in the US National Fungus Collections (BPI).

FIG. 1. Strict consensus of the 600 equally most parsimonious trees resulting from the phylogenetic analysis. Percent bootstrap support values from the 1000 replicates are indicated above branches. GenBank accession numbers are shown to the right of the names.


M. fragariae AY152597
M. fragariae AF297325
M. fragariae AY152596
M. fragariae AY152595
M. fragariae AF173312
R. collo-cygni AF173310
R. sp. AJ417496
M. rubella AY490767
M. punctiformis AY490759 ex Acer
M. punctiformis AY490760 ex Tilia
M. punctiformis AY152593 ex Acer
M. punctiformis AY152594 ex Quercus
M. phacae-frigidae AY490758
M. punctiformis AY490762
M. punctiformis AY490764
M. punctiformis AY490766
M. punctiformis AY490765
M. punctiformis AY490761
M. punctiformis AF222848
M. punctiformis AY490763 ex-epitype
M. nyssicola HQ162263
M. nyssicola HQ162266 ex-epitype
M. nyssicola HQ162265
M. nyssicola HQ162264
M. graminicola AY152602
M. graminicola AY152603
M. graminicola AF181692
M. graminicola AF181693
Septoria passerinii AF181697
Cladosporium bruhnei AY251078
Outgroup
sensu lato
sensu stricto
Ramularia clade
99
55
81
91
61
75
100
70
65
57
99
62
100
91
0.9

DNA isolation, amplification, sequencing

DNA was isolated from cultures on CMD and the ITS region rDNA was amplified and sequenced following the methods of Bao et al. (2010). Sequences were also assembled and edited from chromatographs according to Bao et al. (2010).

Phylogenetic analyses

An initial BLAST search on GenBank indicated that the *Nyssa* isolates were closely related to *M. punctiformis* with 96% maximum identity. The ITS region sequence data from these isolates were subsequently aligned with that of the taxa included in a prior taxonomic study of *M. punctiformis* (Verkley et al. 2004) using Clustal X version 2.0.10 (Larkin et al. 2007). A preliminary analysis employed PAUP* 4.0b10 (Swofford 2002) using the same settings as Verkley et al. (2004). A reduced dataset was constructed using the *Nyssa* isolates and selected members of the *Ramularia* clade and closely related species from the Verkley et al. (2004) study with *Cladosporium bruhnei* Linder as the outgroup ["*Davidiella tassiana*" (De Not.) Crous & U. Braun of Verkley et al. 2004] and aligned with Clustal X using the default settings (Larkin et al. 2007) with the ingroup aligned in the first step and the outgroup added in a subsequent second step alignment. The resulting alignment was visually inspected and the unaligned ends were omitted from later analyses. GenBank accession numbers of included sequences are indicated in Fig. 1. A maximum parsimony analysis was performed using the default parameters of the heuristic search of PAUP* 4.0b10 (Swofford 2002) that included characters 50-560 of the dataset. A parsimony bootstrap analysis was performed using the default settings of PAUP* 4.0b10 (Swofford 2002) with 1000 replicates and maxtrees set at 1000.

Results

The preliminary analysis showed the *Nyssa* isolates were part of the *Ramularia* clade (Verkley et al. 2004, Crous et al. 2009), also known as *Mycosphaerella* sensu stricto (Crous et al. 2009). Analyses of the reduced ITS region dataset contained thirty taxa with *Cladosporium bruhnei* as outgroup and included 501 characters, of which 83 were parsimony informative. The heuristic search of the maximum parsimony analysis produced 600 equally most parsimonious trees of length 225. The consistency (CI), homoplasy (HI), retention (RI), and rescaled consistency (RC) indices were 0.831, 0.169, 0.912, and 0.758, respectively. A strict consensus tree is presented in Fig. 1. The *Ramularia* clade (*Mycosphaerella* sensu stricto) was recovered with 99% bootstrap support (BS). The isolates from *Nyssa* formed a well-supported clade (99% BS). A larger clade, one lacking significant statistical support but composed of several moderately supported subclades, was sister to the *Nyssa* isolate clade. One of these subclades is *M. punctiformis* sensu stricto with 65% BS, which is distinct from the isolates from *Nyssa* that are determined to represent *M. nyssicola*. Isolates of *M. punctiformis* sensu lato and *M. phacae-frigidae* grouped in a clade (75% BS) with *M. phacae-frigidae* sister to a strongly supported (100% BS) *M. punctiformis* sensu lato clade.

Taxonomy

Mycosphaerella nyssicola (Cooke) F.A. Wolf, Mycologia 32: 333. 1940, as '*nyssaecola*'. FIGS. 2–5

≡ *Sphaerella nyssicola* Cooke, Hedwigia 17: 40. 1878, as '*nyssoecola*'.

Lectotypus of *Sphaerella nyssicola* (**hic designatus**): USA. Florida: Gainesville, on leaves of *Nyssa*, leg. Ravenel, FUNGI AMERICANI EXSICCATI no. 96 (BPI *608979*). Note: BPI has more than one of these exsiccatae. This one is mounted on a separate sheet and given a specific BPI number.

Epitypus of *Sphaerella nyssicola* (**hic designatus**): USA. Maryland: Prince George's Co., Glenn Dale, U.S. Plant Introduction Station, 11601 Old Pond Dr. (38°58'00.49"N 76°48'12.78"W), on overwintered, dead, and fallen leaves of *Nyssa* spp., V.2009, leg. RT Olsen (BPI *880897*, AR 4656 ex-epitype culture deposited at CBS 127665, ITS GenBank Accession No. HQ162266).

PSEUDOTHECIA developing overwinter on fallen dead leaves; chiefly hypophyllous; scattered to gregarious, sometimes touching but not confluent; erumpent; semi-immersed; globose; black; 65–125 µm diam. (74–97 µm diam. in lectotype); ostiole apical, 10–23 µm diam. PSEUDOTHECIAL WALL with 2–3 layers of brown textura angularis. PARAPHYSES absent. ASCI bitunicate, 34–48 × 6.5–8 µm, fasciculate, cylindrical, straight to slightly curved, with a short pedicel, octosporous. ASCOSPORES 6.4–8.3 × 1.9–3.2 µm, Q = 2–3.3 µm (^{m} = 7.1 × 2.6 µm, Q ^{m} = 2.7 µm), fusoid-ellipsoid, tapering towards ends, tapering more prominently towards lower end, ends obtuse, widest above septum, with 1 septum, median or slightly supramedian, slightly constricted at septum, thin-walled, hyaline, uniseriate to biseriate, sometimes oblique within and among asci. Germination not observed.

IN CULTURE: COLONY after 31 days on PDA at approx. 22°C and exposed to ambient lighting, up to 29 mm diam., low without abundant aerial mycelium, raised in a mound at center, texture somewhat slimy but not yeast-like, pinkish, or with a pinkish base covered by a whitish tomentum, paler at margin, reverse pinkish to rosette, not sporulating, older cultures sometimes developing fruiting structures more or less globose, black, presumably immature, with walls textura angularis. MYCELIUM with hyphae branching, septate, hyaline or faintly pigmented at times when viewed in mass, walls smooth, width 1.9–3.2 µm.

SPECIMENS EXAMINED: **USA. FLORIDA:** (BPI *608979*, designated lectotype); idem (3 additional exsiccatae duplicates of lectotype without BPI numbers); **GEORGIA: Richmond Co.** AUGUSTA, PHINIZY SWAMP NATURE PARK, on overwintered, dead and fallen leaves of *Nyssa aquatica*, 19.III.2010, leg. J.E. Gordon (BPI *880908*, asci and ascospores not observed); **MARYLAND: Prince George's Co.** GLENN DALE, U.S. PLANT INTRODUCTION STATION, 11601 OLD POND DR., 38°58'00.49"N 76°48'12.78"W, on overwintered, dead and fallen leaves of *Nyssa* spp., V.2009, leg. RT Olsen (BPI *880897*, designated epitype, ex-epitype culture deposited at CBS 127665 AR 4656, ITS GenBank Accession No.

FIG. 2. *Mycosphaerella nyssicola*. UPPER: leaf discoloration and defoliation at U.S. National Arboretum research plots, Glenn Dale, MD. LOWER: leaf spot symptoms on *Nyssa ogeche* in fall, USNA, Washington, DC.

HQ162266; AR 4655 additional culture not preserved, ITS GenBank Accession No. HQ162265); idem (BPI 880800, culture deposited at CBS 127664 = AR 4629, ITS GenBank Accession No. HQ162264; AR 4626 additional culture not preserved, ITS GenBank Accession No. HQ162263); **NORTH CAROLINA:** (BPI *608981*, *876718*); **WEST VIRGINIA:** (BPI *601980*, immature; BPI *608980*, immature).

FIG. 3. *Mycosphaerella nyssicola*. UPPER LEFT: leaf spot symptoms on *Nyssa sylvatica* × *N. ogeche* hybrid in USNA research plots, Beltsville, MD. UPPER RIGHT: leaf spot symptoms on *N. ogeche* and *N. sylvatica* growing at USNA research plots, Washington, DC. LOWER LEFT: leaf spot symptoms on *N. sinensis* growing in the Asian collections at the USNA, Washington, DC. LOWER RIGHT: overwintering *Nyssa* leaves at USNA research plot, Glenn Dale, MD.

ANAMORPH (SPERMATIAL STATE): ***Asteromella nyssae*** (Cooke) Aa, A revision of the species described in *Phyllosticta*: 336. 2002.

≡ *Phyllosticta nyssae* Cooke, Grevillea 12: 26. 1883.

Lectotypus of *Phyllosticta nyssae* (**hic designatus**): USA. Georgia: Darien, on leaves of *Nyssa capitata*, leg. Ravenel, FUNGI AMERICANI EXSICCATI no. 798 (BPI *353790*). Note: BPI has more than one of these exsiccatae. This one is mounted on a separate sheet and given a specific BPI number.

LEAF SPOTS epiphyllous, but discoloring beneath, irregular, frequently coalescing, large, pallid with margin purplish or purplish. SPERMOGONIA chiefly hypophyllous; frequently but not always associated with spots; scattered to

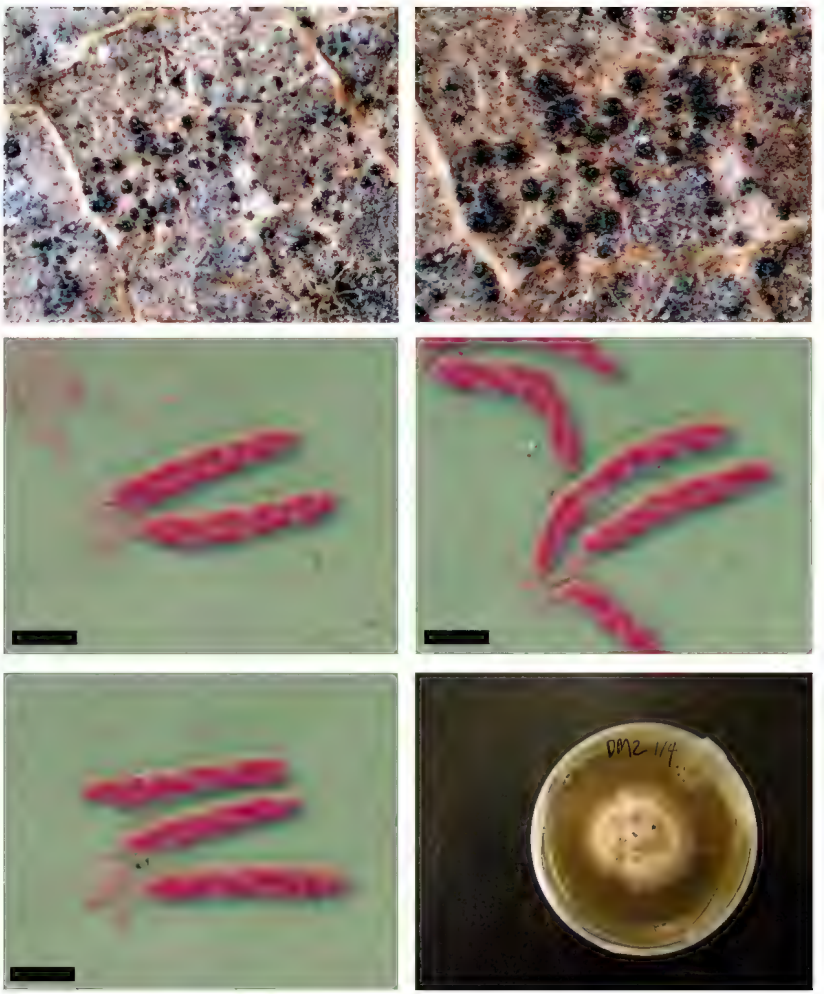


FIG. 4. *Mycosphaerella nyssicola*. UPPER ROW: pseudothecia of BPI *608981*. MIDDLE ROW AND LOWER LEFT: asci and ascospores of BPI *608981*. LOWER RIGHT: ex-epitype culture on PDA (CBS 127666). Scale bars = 10 µm.

gregarious, sometimes touching but not confluent; erumpent; semi-immersed; pseudopycnidial; globose; black; 61–103 µm diam. (65–103 µm diam. in lectotype); ostiole apical, 16–29 µm diam. (16–23 µm diam. in lectotype); unilocular. SPERMAGONIAL WALLS composed of an outer layer of brown textura angularis and a hyaline inner layer that produces the spermatogenous cells. SPERMATOGENOUS CELLS determinate; phialidic; hyaline; walls smooth; discrete or more typically integrated on spermatiophores; spermatiophores up to three

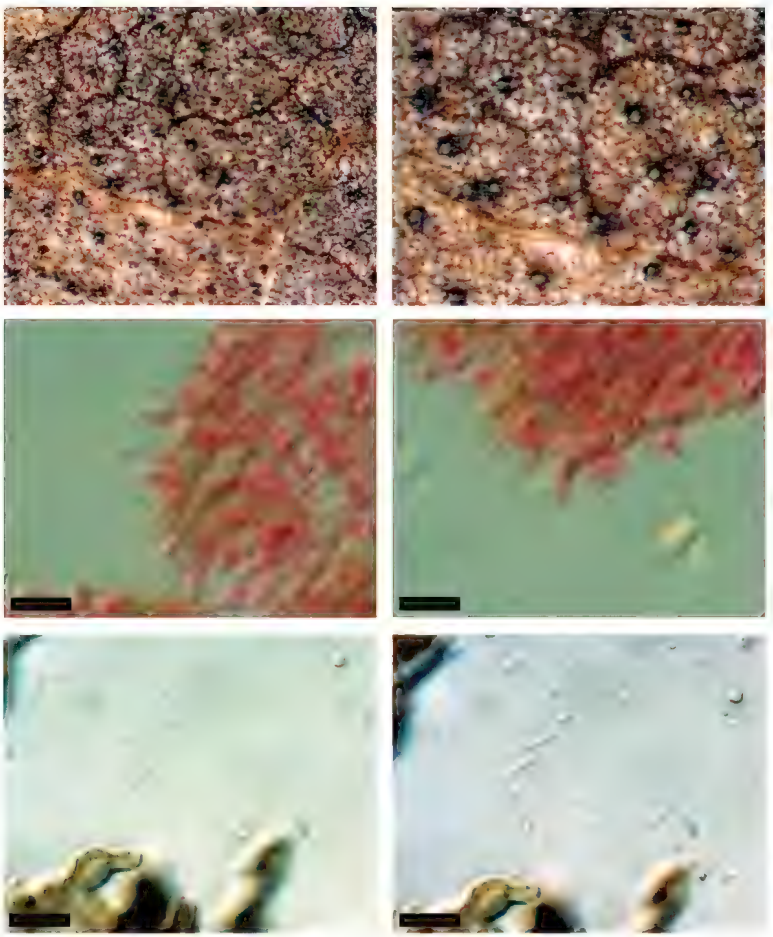


FIG. 5. *Asteromella nyssae* (spermatial state of *Mycosphaerella nyssicola*). UPPER ROW: spermogonia of BPI *353792*. Middle row: conidiogenous cells of BPI *353799*. LOWER ROW: spermatia of BPI *353792*. Scale bars 10 µm.

septate, occasionally branching at base; loci apical or on short side branches formed one per cell immediately below septa of spermatiophores. SPERMATIA 3.2–5.8 × 0.6–1.6 µm, Q = 2.4–5.3 µm (^{m} = 4.1 × 1.2 µm, Q ^{m} = 3.4 µm) (3.8–4.5 × 1–1.3 µm in lectotype), at times copious and adhering in large numbers, bacilliform, aseptate, walls smooth, hyaline, eguttulate.

SPECIMENS EXAMINED: **USA. GEORGIA:** (BPI *353789*, duplicate of lectotype; BPI *353790*, designated lectotype); **MICHIGAN:** (BPI *353793*); **NORTH CAROLINA:** (BPI *353792*); **SOUTH CAROLINA:** (BPI *353796*, *353797*); **TENNESSEE:** (BPI *860755*); **VIRGINIA:** (BPI *353788*, *353791*, *353795*, *353799*); **WEST VIRGINIA:** (BPI *353798*, *521392*, *521393*).

Habitat & Distribution — Based on Farr & Rossman (2010) and examined specimens, *Mycosphaerella nyssicola* is known to occur on leaves of *Nyssa aquatica*, *N. biflora* (≡ *N. sylvatica* var. *biflora*), *N. ogeche*, and *N. sylvatica*, and it is widely distributed in the eastern USA (AL, FL, GA, MD, MI, NC, NY, PA, SC, TN, VA, WV), especially in the southeastern states. Observations of disease symptoms at the USDA field plot also confirmed as hosts: *N. sinensis* and two F1 hybrids, *N. sylvatica* × *N. ogeche* and *N. sylvatica* × *N. sinensis*. It is possible that the distribution of *M. nyssicola* is greater than that reported in the literature, and the fungus may occur throughout the range of *Nyssa* in the USA.

Comments — Analyses of the DNA sequence data from the ITS region suggest that isolates from *Nyssa* are distinct from isolates of *M. punctiformis* both sensu stricto and sensu lato as presented by Verkley et al. (2004). Morphological examination of new collections and historical herbarium collections indicates that the *Nyssa* isolates are identical with *Mycosphaerella nyssicola*. Although *M. nyssicola* is more or less indistinguishable from *M. punctiformis*, as was noted by Aptroot (2006), DNA evidence and host specificity support *M. nyssicola* as a distinct species on *Nyssa*.

With respect to types, Cooke (1878) described *Sphaerella nyssicola* as a new species occurring on *Nyssa*, and the original material was distributed as exsiccatae in Ravenel's Fungi Americani Exsiccati. Confusingly, the labels of the exsiccatae cited in the protologue by Cooke (1878) indicate specimens from Florida on *Nyssa*, while the protologue lists South Carolina and *Nyssa multiflora* (= *N. sylvatica*) for distribution and host. These exsiccatae are syntypes (McNeill et al. 2006: Art. 9.4). Aptroot (2006), who listed a specimen from South Carolina at K as holotype and exsiccata no. 96 at BPI from Florida as isotype, did not use the phrase 'hic designatus,' which would have caused an accidental lectotypification of the species (McNeill et al. 2006: Arts. 7.11, 9.8). However, to assume that a collection housed in the original author's herbarium represents the holotype when no holotype has been designated is not the correct course of action. Additional confusion results from whether original material exists from Florida, from South Carolina, or from both regions. To resolve the confusion, we designate one syntype as lectotype (McNeill et al. 2006: Art. 9.2). The original material (as noted by Cooke, 1878) is immature. Despite repeated attempts, we were unable to find asci or ascospores in any of the four exsiccatae (including the designated lectotype) housed at BPI.

Similarly, Cooke (1883) described *Phyllosticta nyssae* as a new species on *Nyssa capitata* (= *N. ogeche*), and the original material was distributed as exsiccatae in Ravenel's Fungi Americani Exsiccati. Seaver (1922), Wolf (1940), and Aa & Vanev (2002), all of whom provided morphological data on this species and cited the exsiccatae, did not designate a type. Hence, we here also designate one syntype as lectotype.

Lastly, because the type material is immature, we epitypify *S. nyssicola* based on a recent collection of *Mycosphaerella nyssicola* from Maryland. The living culture and DNA data obtained from the epitype aid in the application of the name (McNeill et al. 2006: Art. 9.7), although we are somewhat reluctant to epitypify the name based on a specimen that was not collected in the type locality (i.e., either Florida or South Carolina). The epitype is otherwise identical in all regards to material observed by Wolf (1940) and so maintains current usage of the name.

Wolf (1940) provided an excellent symptomatological account of this leaf spot disease and the life history of *M. nyssicola*. We have nothing to add except that Koch's postulates have not yet been completed, linkage of the teleomorphic and spermatial states is circumstantial, and no attempts have been made to germinate spermatia that would confirm they are not conidial in nature. We also have not studied fresh material of the spermatial state. As with Wolf (1940), we found no *Ramularia* or other conidial state for this fungus. *Mycosphaerella nyssicola* is typical of members of the *Ramularia* clade (also known as section *Mycosphaerella* or *Mycosphaerella* sensu stricto) and it is quite similar to *M. punctiformis* (Verkley et al. 2004, Crous 2009, Crous et al. 2009). At this time, we do not propose a new combination in *Ramularia* based on the teleomorphic state as advocated by Crous et al. (2009) for species of *Mycosphaerella* sensu stricto, as the latter generic name correctly adheres to ICBN Art. 59.1 (McNeill et al. 2006).

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Acaulospora soloidea, a new arbuscular mycorrhizal fungus from rhizosphere soils of *Murraya paniculata*

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Abstract — A new species of *Acaulospora* from the laterite soils in Goa, India is described and illustrated. The spores have a unique wall with fibrillose, hairy outgrowths, which appear like the rays of the sun when mounted in water or PVLG, hence named as *A. soloidea*.

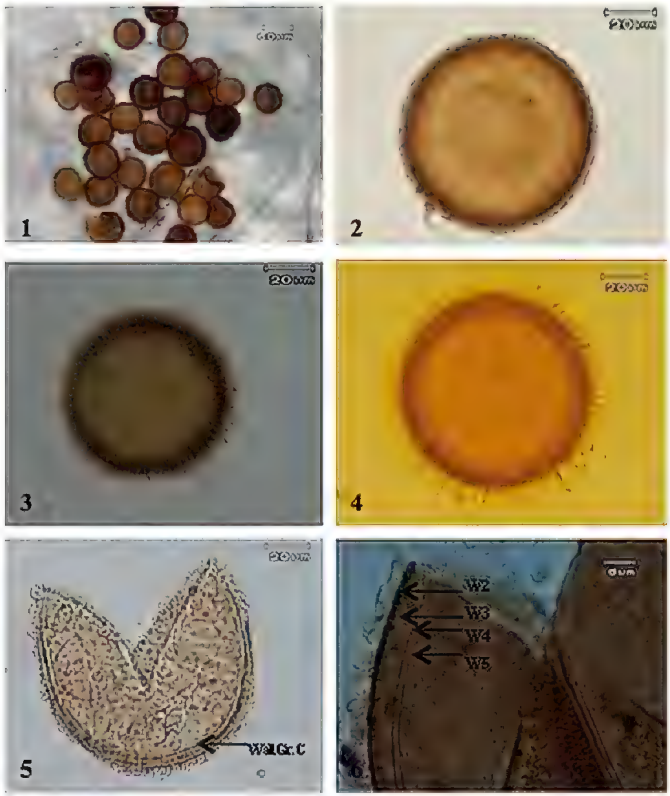
Key words — *Glomeromycota*, spore morphology, taxonomy, species description

Introduction

A survey was conducted on the rhizosphere soils of ornamental flowering plants of Goa state to collect the arbuscular mycorrhizal fungal diversity and distribution data. During the survey an interesting taxon was isolated from *Murraya paniculata*. The spore wall is covered with numerous fibrillose, acellular, hairy outgrowths forming a thick cover. When the spores are mounted in a drop of water or PVLG, these hairs unfold from the spore surface to spread around the spore like solar rays, giving the spore an appearance of sun, hence proposed as *Acaulospora soloidea* sp. nov.

Materials & methods

Spores were isolated from the rhizosphere soils by wet sieving, decanting and sucrose centrifugation (Sieverding 1991). The spores were mounted in Polyvinyl alcohol lacto-glycerol (PVLG) and in PVLG mixed with Melzer's reagent (1:1, v/v). Wall description and terminology are based on those suggested by Walker (1983) and Walker & Vestberg (1998). Type material has been deposited in the Goa University Arbuscular Mycorrhizal Culture Collection. To study more carefully the external morphology of the spores, a number of them were prepared for Scanning Electron Microscopy (SEM). Before the preparation of the material for SEM, spores were put under a dissecting microscope to remove any debris adhering to the spore surface. For SEM studies, clean dry spores were mounted on an aluminum stub with double-sided transparent tape, coated with gold, and observed.



Figs 1–6. *Acaulospora soloidea*. 1. Fresh spores under transmitted light. 2. Dry spore. 3. Spores mounted in PVLG. 4–6. Spores showing wall layers.

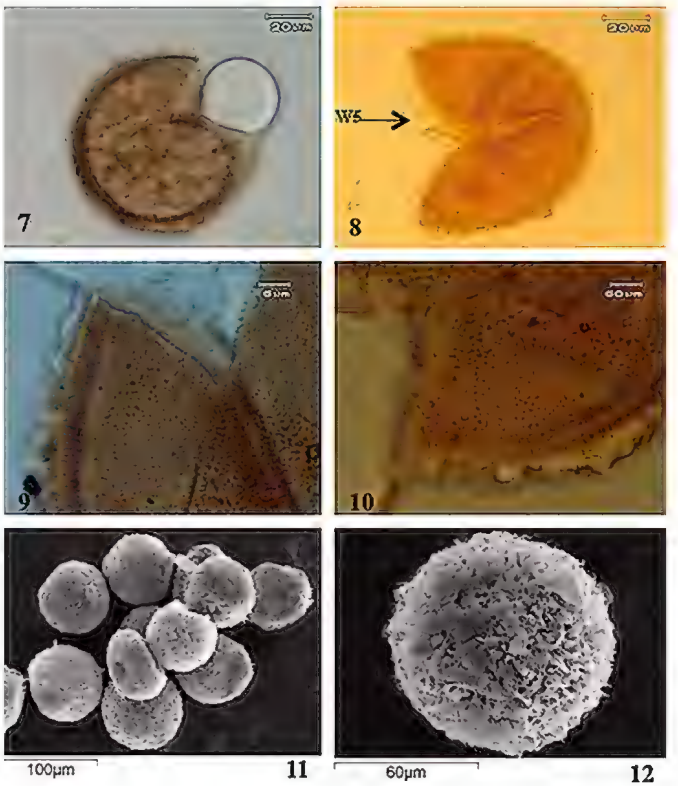
Acaulospora soloidea Vaingankar & B.F. Rodrigues, **sp. nov.** Figures 1–12

Mycobank MB 518836

Sporae singulae pallide brunneae vel brunneae, globosae vel subglobosae (50–)65–84(–90) µm diam. Tunica sporae e stratis quinque in turmis tribus, in totum 2.0–3.0 µm crassa. Tunica exterium (stratum 1) unitum, hyalinum 0.4–0.6 µm crassa, caducus, tenue et evanescens. Tunica media stratis duobus, stratum exterius (stratum 2) brunneae, 1.2–1.6 µm crassa, rigido, cum appendicibus numerosis fibrillosis, pilosae sporae, 3–6 µm cr. formantibus. Stratum interioris (stratum 3) hyalinum vel pallide brunneae 0.3–0.4µm crassa. Tunica interior stratis duobus (strati 4, 5); quarto hyalinum, 0.1–0.2 µm crassa; quinque hyalinum, 0.1–0.2 µm crassa amorpho.

Holotype: India. Goa: Arambol, from rhizosphere soil of *Murraya paniculata* (L.) Jack (*Rutaceae*), 3 Jan. 2009, Jyoti. (Arbuscular Mycorrhizal Culture Collection, Goa University: Slide no. OFP-VJ1).

Etymology: *soloidea*, from Latin *sol* (sun) and *oideus* (resembling), referring to the sun like appearance of the spore.



Figs 7–12. *Acaulospora soloidea*. 7. Crushed spore showing lipid content. 8. Spore mounted in PVLG + Melzer's reagent showing inner wall. 9–10. Spores showing hairy ornamentation. 11–12. SEM images of spores.

Spores formed singly in the soil, sessile, borne laterally on the subtending hypha of the sporiferous saccule. The spores are pale brown to brown, globose to subglobose (50–)65–84(–90) µm diam. Spore wall structure of 5 walls (1–5) in three groups (A, B, C), exterior, medium and interior, in total 2.0–3.0 µm thick (Figs 4–6, 8). Group A with a single wall (W1). Wall 1 is hyaline, 0.4–0.6 µm thick, sloughing completely, evanescent and thus present only in young spores. Group B consisting of two walls (W2 and W3). Wall 2 is continuous, brown 1.2–1.6 µm thick, rigid, with numerous acellular, fibrillose, hairy outgrowths forming a pile or thick coat 3–6 µm on the spore surface (Figs 3–4). The hairs are tightly packed together on the spore surface when dry (Fig. 2). The length of each may vary from 10–15 µm, Wall 3 hyaline to light brown, laminated, 0.3–0.4 µm thick tightly adherent to W2. Group C composed of two hyaline flexible walls (W4 and W5), which are formed after the differentiation of spore

wall. Wall 4 hyaline, bilayered, very thin 0.1–0.2 µm, does not show any reaction with Melzer's reagent. Wall 5 is hyaline, thin, 0.1–0.2 µm, amorphous and is not beaded (Fig. 8). Spore contents of hyaline lipid globules (Fig. 7).

Discussion

Gerdemann & Trappe (1974) established the genus *Acaulospora* and presented the first key for the two species, *A. laevis* and *A. elegans*. The key differentiated one species known to produce spores with a smooth surface (*A. laevis*) from another with an ornamented surface (*A. elegans*). Presently 19 *Acaulospora* species with smooth spore surfaces (http://www.lrz-muenchen.de/~schuessler/amphylo/) and 15 *Acaulospora* species with outer spore wall ornamentation have been identified (Sieverding et al. 2006). Schenck et al. (1984) presented a key to the ornamented species of *Acaulospora*. Differentiating features such as spines, tubercles, ridges, folds, pits or cracks as for the spore wall ornamentations were used. The species described here is included in this genus. It presents affinities with the type species in spore shape and wall layers. It differs from other *Acaulospora* species in the morphology of the spore surface, which gives rise to numerous acellular, fibrillose hairy outgrowths forming a pile or thick coat on the spore surface, which when extended look like the rays of the sun. On the basis of its morphological characteristics, the isolate represents a novel species of the genus *Acaulospora*, for which the name *A. soloidea* sp. nov. is proposed. *Acaulospora soloidea* can be easily distinguished from all other known species in the genus.

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Conidial fungi from the semi-arid Caatinga biome of Brazil. New species and records for *Thozetella*

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Abstract As a result of an investigation of microfungi in northeastern Brazil, the hyphomycete *Thozetella submersa* sp. nov. is described from submerged wood. Its diagnostic features are the smooth, elliptic-fusiform, obclavate to rarely obovoid to obclavate, slightly curved, sometimes corniculate at the ends, microawns. *T. boonjiensis* and *T. gigantea* are recorded for the first time for the American continent. A key to all described species is included. A synopsis of drawings of microawns (most based on original papers) is provided for all *Thozetella* species.

Key words anamorphic fungi, diversity, taxonomy

Introduction

Thozetella Kuntze presently includes fifteen species (Castañeda et al. 2002, Paulus et al. 2004, Allegrucci et al. 2004, Jeewon et al. 2009) found in temperate and warm regions (Nag Raj 1976, Grandi & Gusmão 2002, Piontelli & Giusiano 2004, Barbosa et al. 2007). Among the variety of sterile structures known for the hyphomycetes, the presence of microawns is unique for the genus (Sutton & Cole 1983).

Recent phylogenetic and morphological research of five species of *Thozetella* from Australia by Paulus (et al. 2004) confirms that *Thozetella* species are anamorphs of the ascomycete *Chaetosphaeria* Tul. & C. Tul. Their key to 14 described species of *Thozetella* (Paulus et al. 2004) did not include *T. buxifolia* Allegr. et al. (Allegrucci et al. 2004). In their synoptic table of *Thozetella* species, Paulus et al. (2004), who apparently did not examine the holotype, cited a median to submedian septum for microawns in *T. canadensis* Nag Raj although this character was not mentioned in the protologue (Nag Raj 1976). In the present work, almost all characters are based on original species descriptions.

Recently, *T. pinicola* S.Y.Q. Yeung et al. was described from leaf litter of *Pinus elliottii* Engelm. in Hong Kong. This new taxon was based on morphology and DNA sequence analyses (Jeewon et al. 2009).

Paulus et al. (2004), Allegrucci et al. (2004), and Jeewon et al. (2009) retain *T. ciliata* (R.F. Castañeda et al.) Hol.-Jech. & Mercado within *Thozetella*. However, we refer this specimen to *Venustosynnema ciliatum* (R.F. Castañeda et al.) R.F. Castañeda & W.B. Kendr. In their discussion of *V. ciliatum*, Castañeda-Ruiz et al. (2002) note that the absence microawns and presence of dark brown setae are characters that exclude the species from *Thozetella*.

Thozetella species have been reported from leaf litter (Grandi 1999, Parungao et al. 2002, Castañeda-Ruiz et al. 2003, Allegrucci et al. 2004), decaying floral parts (Agnihothrudu 1958), soil (Agnihothrudu 1962), roots (Waipara et al. 1996), stalks (Nag Raj 1976), and bark (Morris 1956). This constitutes the third report of the genus from freshwater habitats following those of *T. havanensis* R.F. Castañeda (from submerged leaf litter) and *T. nivea* (Berk.) Kuntze (from wood) (Sivichai et al. 2002, Delgado-Rodrigues & Mena-Portales 2004).

From Brazil, five *Thozetella* species have been found: *T. cristata* Piroz. & Hodges, *T. cubensis* R.F. Castañeda & G.R.W. Arnold, *T. havanensis* (Barbosa et al. 2007, Silva & Grandi 2008), *T. queenslandica* B.C. Paulus, Gadek & K.D. Hyde (Cruz & Gusmão 2009), and *T. tocklaiensis* (Agnihothr.) Piroz. & Hodges (Maia et al. 2002).

Materials & methods

Study site. Collecting trips were made to the hygrophilous forests called "Serra da Jibóia" in the semi-arid region in the northeastern of Brazil. This area has been described previously (Barbosa et al. 2007, Marques et al. 2007)

Collection techniques. Submerged leaves and wood debris were collected from a lotic habitat in an unnamed stream in the "Serra da Jibóia". Samples were placed in plastic bags and returned to the laboratory. The plant material was then incubated at 25°C in Petri dish moist chambers and stored in 50 L plastic boxes with 200 ml sterile water plus 2 ml glycerol. Samples were examined over four weeks for the presence of conidiomata.

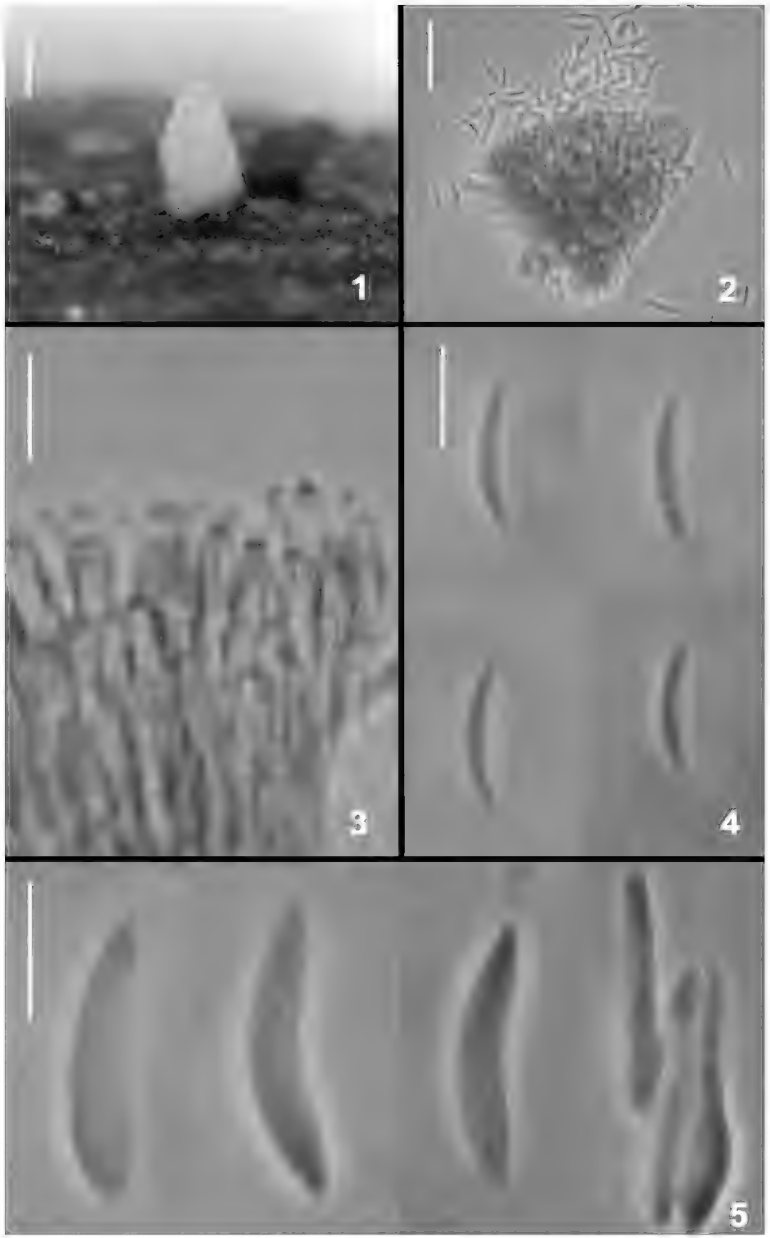
Specimen examination. Conidiomata were located with a dissecting microscope and removed to a glass slide where they were crushed and mounted in polyvinyl alcohol-glycerol (8.0 g in 100 ml of water, plus 5 ml of glycerol). Measurements were made of fixed material. Permanent slides were deposited in HUEFS.

Taxonomy

Thozetella submersa F.R. Barbosa & Gusmão, **sp. nov.** Fig. 1–5

MycoBank MB518831

Ab omnibus speciebus Thozetellae differt microaristis unicellularibus ellipsoidi-fusiformibus, raro obovatis vel obclavatis, minute curvis, laevibus, utrinque interdum solo corniculatis praeditis, hyalinis, 16 25µm longis, 3 4 µm latis at basi, 2 3 µm latis at apicem.



FIGS. 1–5. *Thozetella submersa* (from the holotype). 1. Conidioma on the natural substrate. 2. General aspect of a conidioma without the conidial mass. 3. Conidiogenous cells. 4. Conidia. 5. Microawns. (Scale bars: 1 = 100 µm; 2 = 50 µm; 3–5 = 10 µm).

HOLOTYPUS: Brazil. Bahia: Santa Terezinha, Serra da Jibóia, on submerged wood from a stream, 25.III.2009, coll. FR Barbosa, HUEFS 141560.

ETYMOLOGY: the Latin *submersa* refers to the submerged habitat.

CONIDIOMATA sporodochial, superficial, sessile, 200–250 × 105–150 µm, moist and white mass. CONIDIOPHORES macronematous, compact at the base but more or less free distally, septate, smooth, cylindrical, brown, paling toward the apex, ≤ 3 µm diam. CONIDIOGENOUS CELLS monophialidic, integrated, determinate, terminal, cylindrical, light brown, 7.5–10 × 2.5–3 µm, lacking an apical collarette. MICROAWNS not visible in mass on the natural substratum, smooth, elliptic-fusiform, rarely obovoid to obclavate, slightly curved, sometimes corniculate at the ends, hyaline, 16–25 µm long, 3–4 µm wide at the base, 2–3 µm wide at the apex. CONIDIA aseptate, smooth-walled, finely guttulate or eguttulate, lunate, hyaline, 14–15 × 2–2.5 µm, provided with a single filiform setula at each end, 5–7 µm long.

NOTES: *Thozetella submersa* can be easily diagnosed by the morphology and smaller size of the microawns. A synopsis of microawns (FIG. 6) and a key to the published species are included.

Thozetella boonjiensis B.C. Paulus, Gadek & K.D. Hyde, Mycologia 96: 1076. 2004.

CONIDIOMATA sporodochial. MICROAWNS L-shaped, 0-septate, hyaline, 54–63 × 3–4.5 µm, apex acerose. CONIDIA 10.5–14.3 × 1.5–3 µm. SETULAE 5.3–7.5 µm long.

EXAMINED MATERIAL: BRAZIL. BAHIA: Santa Terezinha, Serra da Jibóia, on decaying wood, 01.VIII.2008, coll. Fiuza P.O. (HUEFS148834).

NOTES: *Thozetella boonjiensis* resembles *T. acerosa* B.C. Paulus et al., *T. gigantea,* and *T. tocklaiensis* in the L-shaped microawns, which are, however, septate in *T. acerosa.* Both microawns and conidia are larger in *T. gigantea* and smaller in *T. tocklaiensis* (Paulus et al. 2004, Piontelli & Giusiano 2004). *Thozetella boonjiensis* was previously known only from the type locality (Australia) (Paulus et al. 2004).

Thozetella gigantea B.C. Paulus, Gadek & K.D. Hyde, Mycologia 96: 1080. 2004.

CONIDIOMATA sporodochial; MICROAWNS L-shaped, 0-septate, hyaline, 81–141 µm long, 4.5–6 µm wide at base. CONIDIA 13–15 × 1.5–2 µm. SETULAE 7.5–9 µm long.

EXAMINED MATERIAL: BRAZIL. BAHIA: Santa Terezinha, Serra da Jibóia, on decaying leaves, 02.VIII.2007, coll. Silva, S.S. (HUEFS141569).

NOTES: *T. gigantea* is characterized by its microawns, which are the largest for the genus (Paulus et al. 2004). This species has been registered only from type locality (Australia) (Paulus et al. 2004).

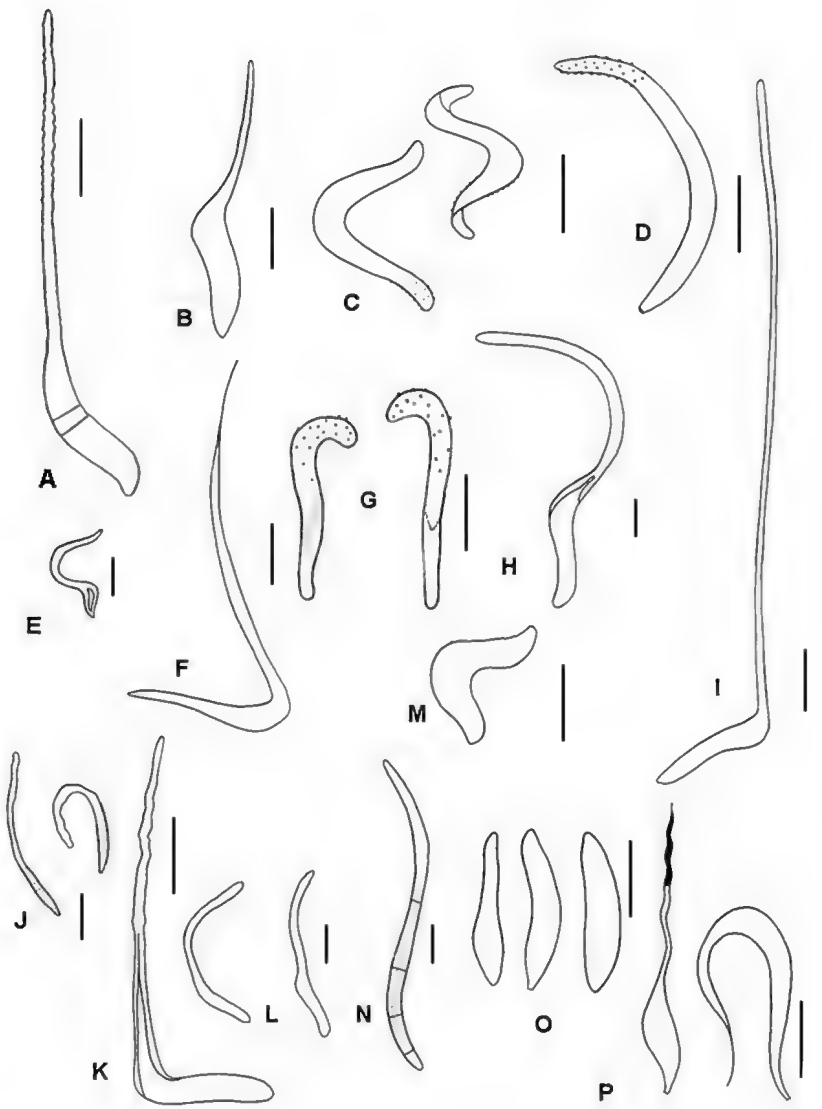


FIG. 6. Microawns of *Thozetella* species. A. *T. acerosa*; B. *T. boonjiensis*; C. *T. buxifolia*; D. *T. canadensis*; E. *T. cristata*; F. *T. cubensis*; G. *T. effusa*; H. *T. falcata*; I. *T. gigantea*; J. *T. havanensis*; K. *T. nivea*; L. *T. pinicola*; M. *T. queenslandica*; N. *T. radicata*; O. *T. submersa*; P. *T. tocklaiensis*.
(Scale bars: A P 10 µm).

Key to *Thozetella* species

1. Microawns predominantly L-shaped 2
1a. Microawns not L-shaped or variously shaped 5
2. Microawns 0–2 septate ..... *T. acerosa*
2a. Microawns aseptate 3
3. Microawns 75 µm or longer ..... *T. gigantea*
3a. Microawns shorter than 75 µm 4
4. Microawn apex undulating to geniculate ..... *T. nivea*
4a. Microawn apex straight to slightly undulating ..... *T. boonjiensis*
5. Microawns predominantly sickle-shaped, uncinate, hamate or otherwise strongly curved 6
5a. Microawns predominantly straight, sigmoid or of other shape 11
6. Conidiomata predominantly synnematous 7
6a. Conidiomata predominantly sporodochial 10
7. Synnemata proliferating conidiophores form ridges 8
7a. Synnemata non-proliferating 9
8. Microawns 40–60 × 2.5–3 µm, smooth ..... *T. cristata*
8a. Microawns 25–30 × 3–3.5 µm, verrucose ..... *T. buxifolia*
9. Microawns 40–95 × 2.5–5 µm, setulae 5–8 µm long ..... *T. falcata*
9a. Microawns 30–60 × 3–4.5 µm, setulae 5 µm long ..... *T. radicata*
10. Microawns 21–34 × 2–4 µm, smooth or apically verrucose ..... *T. queenslandica*
10a. Microawns 40–100 µm long, 2.5–4 µm wide at base, 0.5–1 µm wide at apex, smooth ..... *T. cubensis*
11. Conidiomata effuse ..... *T. effusa*
11a. Conidiomata otherwise 12
12. Conidiomata sporodochial 13
12a. Conidiomata synnematous 15
13. Microawns verrucose ..... *T. canadensis*
13a. Microawns smooth 14
14. Microawns elliptic-fusiform, 16–25 × 3–4 wide at the base, 2 3 µm wide at the apex ..... *T. submersa*
14a. Microawns straight or slightly undulating with the apical end acerose, 25–55 × 2.5–5 µm ..... *T. pinicola*
15. Microawns variously shaped, bulbous base, acerose apex, straight, undulate, uncinate or bent,18–38 × 1.5–4 µm ..... *T. tocklaiensis*
15a. Microawns with + uniform width, sigmoid, allantoid, uncinate, 22.4–35 × 1.5–3.2 µm ..... *T. havanensis*

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Chrysothrix flavovirens, Lepraria elobata, and *Ochrolechia arborea* new to Portugal

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Abstract — *Chrysothrix flavovirens, Lepraria elobata,* and *Ochrolechia arborea* are reported as new to Portugal, based on surveys carried out in pine forests along the Portuguese coast. Data on the distribution, secondary products and ecology of the species are presented.

Key words — lichens, epiphytic, sand dunes

Introduction

The epiphytic lichen flora of pine forests in coastal areas in Portugal is poorly studied and is in need of a thorough survey. Pine forests are a common biotope along the Portuguese coast and many of them are classified as Natural Parks or Nature Reserves (Fig. 1A&B: 3, 8, 18–19, 21–22, 24), while others are national forests or forest perimeters that are owned and/or partially managed by the Portuguese State (Fig. 1A&B: 1, 7, 9–18, 20, 24). Moreover, many of them are currently classified as Natura 2000 sites (ICN 2006) by the European Commission (Fig. 1A&B: 1–3, 8, 10, 12, 18–19, 21–22, 24). The only known inventory of epiphytic lichen species in pine forests on sand dunes was generated by Catarino et al. (1985) in the framework of biomonitoring studies.

In the course of our investigations on the epiphytic lichens of these areas — particularly the well-preserved Dunas de Quiaios (Figueira da Foz) (Fig. 1A&B: 12) — new epiphytic lichen records were reported for the Iberian Peninsula (Rodrigues et al. 2007) and a new *Lecanora* species has been published (Rodrigues et al. 2011). These discoveries point to the importance of coastal

pine forests as habitats for lichens, which is an extra incentive to ensure their future preservation. At Dunas de Quiaios, the tree vegetation, rich in epiphytic lichens, is a planted *Pinus pinaster* forest mixed with *P. pinea, Acacia longifolia, A. melanoxylon,* and *Eucalyptus globulus. Arbutus unedo* and *Myrica faya* are also appropriate supports for lichens (Almeida 1997, Danielsen 2008, Rodrigues et al. 2007, 2011).

The aim of this work is to report three crustose and sorediate epiphytic lichens growing on *P. pinaster* and *P. pinea*, new to Portugal: *Chrysothrix flavovirens*, *Lepraria elobata* and *Ochrolechia arborea.* Data on their distribution along the Portuguese coast, secondary products, and ecology are also provided.

Materials & methods

Unless otherwise stated, all specimens were collected by the first author (SAR) on *P. pinaster* trunks in pine forests on sand dunes along the Portuguese coast (Fig. 1A&B, see also EXAMINED SPECIMENS). Samples were deposited in AVE, BG, and LEB herbaria. Distribution maps are based on UTM grid projection and coordinates of the locations are given in MGRS. Maps [A] and [B] represent the distribution along the Portuguese coast of *C. flavovirens*, and *O. arborea* respectively. The location where *L. elobata* was found [12] appears on both maps. Visited locations (indicated by numbers on each map) were geo-referenced using GoogleEarth and maps were plotted using ArcGis version 9.2.

Samples were analysed morphologically and chemically, with standard identification methods for lichenized fungi (Nash et al. 2001, Smith et al. 2009) and compared to authentic material deposited in BG herbarium. Morphology of the thallus was examined with a Leica MS5 stereomicroscope and anatomical observations of hand cut sections of apothecia were performed under a Leitz HM-LUX 3 microscope. For that, sections were mounted in distilled water and K/I and the latter was used for measuring anatomical characters. Chemical analyses followed standardized TLC methods (White & James 1985; Orange et al. 2001). Data regarding companion species were retrieved at Dunas de Quiaios (Fig. 1A&B: 12) during a small biomonitoring campaign, using the method proposed by Asta et al. (2002), in which 9 sampling units with 10 or 12 trees each were studied. Only *C. flavovirens* was detected using this method, and therefore it is the only species for which such data are provided. Only species present in each 10 × 10 cm quadrate where *C. flavovirens* was detected were considered as companion species.

The species

Chrysothrix flavovirens Tønsberg — Fig. 1A

This common species is characterized by a vivid yellowish-green and leprose thallus, which is neither stratified nor lobed. The soralia are discrete to contiguous, forming an areolate or, more often, a thin to thick, continuous thallus. In our material, diffractaic and rhizocarpic acids and ± unidentified substances were detected. Fertile material was found at Mata do Camarido (Caminha) (Fig. 1A: 1). Fertile specimens had scattered and sessile apothecia,

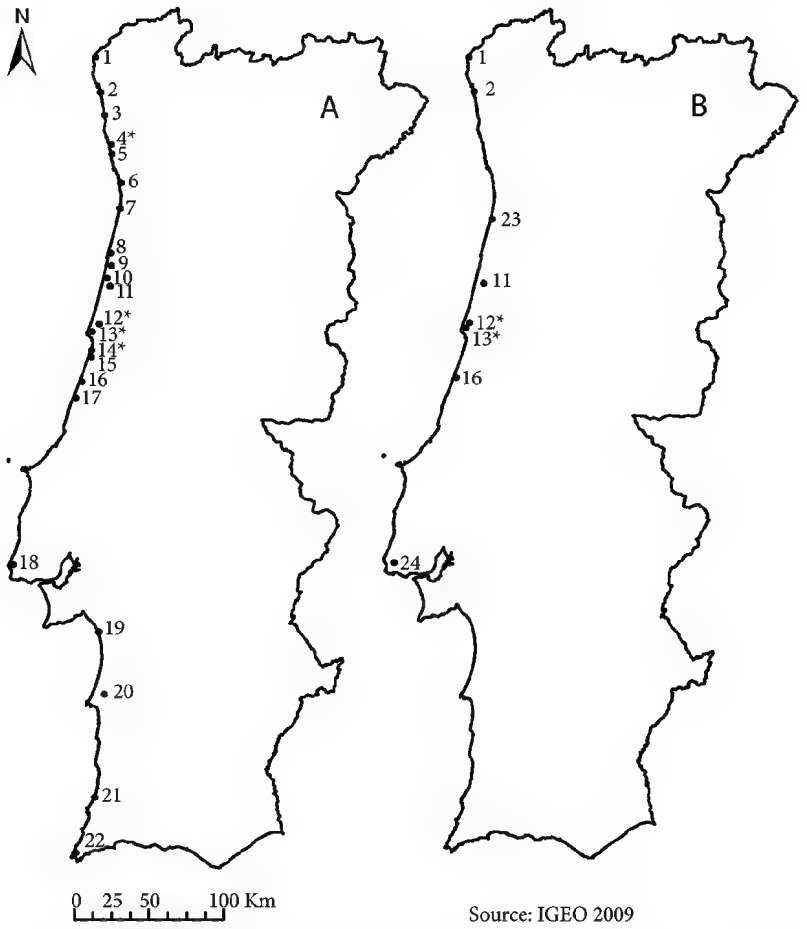

FIG. 1. Distribution of *Chrysothrix flavovirens* [A] and *Ochrolechia arborea* [B] along the Portuguese coast; *Lepraria elobata* [12] appears in both [A] and [B]. Dot numbers correspond to the localities shown in square brackets within the SPECIMENS EXAMINED sections. Asterisks flag locations where the species were found on both *Pinus pinaster* and *P. pinea*.

which were 0.3–0.6 mm in diam. (n = 30), yellowish brown in colour, mostly rounded, convex and immarginate, but sometimes surrounded by soredia as also reported by Laundon (1981). Sections of the apothecia reacted K+ violet in the centre and intensified yellow at the border of the section. The epihymenium, hymenium, and hypothecium were constituted by anastomosing paraphysis, and reacted K/I+ blue. The epihymenium was 2.5–12.5 µm (n = 15) high, the hymenium 38–83.5 µm (n = 8) and the hypothecium 148.5–265 (n = 8). Asci were clavate, sometimes slightly curved, 23–40.5 × 8.5–14 µm (n = 14). Spores

were narrowly ellipsoid, some slightly curved, mostly 3-septate, 9.5–12.5 × 2–4 µm (n = 21), although some were found 2-septate and 1-septate, but of smaller sizes. Detailed descriptions can be found in Laundon (1981), Smith et al. (2009), and Tønsberg (1992).

Habitat and Distribution — *Chrysothrix flavovirens* may form large patches extending over a large part of the pine trunk or appear as small patches associated with other species. At Dunas de Quiaios (Fig. 1a: 12) it was found together mostly with *C. candelaris* (L.) J.R. Laundon, *Hypogymnia physodes* (L.) Nyl., and *Pyrrhospora quernea* (Dicks.) Körb. but also with *Usnea rubicunda* Stirt., *U. subscabrosa* Nyl. ex Motika, and *Flavoparmelia caperata* (L.) Hale, *Lecanora strobilina* (Spreng.) Kieff., *Parmotrema reticulatum* (Taylor) M. Choisy and *Trentepohlia* sp.

This species was frequently found in well-preserved to open coastal pine forests on sand dunes and also in localities with scanty pines, growing on *P. pinaster* and in some sites also on *P. pinea* (Fig. 1a, see also examined specimens). It was also collected in mountains near the coast at Serra da Boa Viagem (Figueira da Foz) (Fig. 1a: 13) and Serra de Sintra (Sintra) (Fig. 1a: 18). Elevations observed ranged from about 2 m at Dunas de Ofir/Fão (Esposende) (Fig. 1a: 3) to about 260 m at Serra de Sintra. Although *C. flavovirens* is widespread throughout the Atlantic and Mediterranean biogeographic regions of Portugal (ICN 2006), it was not found in surveys at a coastal pine forest in south-eastern Portugal, Dunas de Vila Real de Santo António (Vila Real de Santo António). This species, which is frequent and of which reports are increasing, is regarded as a preferentially coastal species of acidic bark (Laundon 1981 [as sorediate thalli of *C. chrysophthalma* (P. James) P. James & J.R. Laundon], Kowalewska & Jando 2004, Tønsberg 1992 [as sorediate thalli of *C. chrysophthalma*], Smith et al. 2009).

Chrysothrix flavovirens is widely distributed in Europe (Aptroot et al. 2003; Kowalewska & Jando 2004; Laundon 1981; Llimona & Hadlun 2001; Nimis & Martellos 2008; Smith et al. 2009; Søchting & Alstrup 2008; Sparrius et al. 2002; Suija et al. 2009; Tønsberg 1992, 1994) and is also known from North America (Richardson et al. 2009) and Asia (Kowalewska & Jando 2004). A very similar species recently described from eastern North America, *C. chamaecyparicola* Lendemer, is similar to *C. flavovirens* except that it lacks diffractaic acid (Lendemer & Elix 2010).

Specimens examined (square bracketed numbers refer to localities indicated in Fig. 1a): **PORTUGAL. Minho:** [1] **Caminha.** Moledo, Mata Nacional do Camarido, 29TNG1133, 14 m, 22.3.2009, *AVE-L 337, LEB-Lichenes 7810.* [2] **Viana do Castelo.** Chafé, Praia da Amorosa, 29TNG1510, 17 m, 5.9.2009, *AVE-L 380, LEB-Lichenes 7811.* [3] **Esposende.** Fão, Dunas de Ofir/Fão (Parque Natural do Litoral Norte), 29TNF1894, 2 m, 15.5.2009, *AVE-L 358.* **Douro Litoral:** [4] **Vila do Conde.** Mindelo, Reserva Ornitológica do Mindelo, 29TNF2275, 24 m, 25.6.2009, *AVE-L 376, LEB-Lichenes 7812;* id., on trunk of *Pinus pinea AVE-L 364.* [5] **Matosinhos.**

Angeiras, Parque de Campismo de Angeiras — in a small pine stand on sand dunes used for camping, 29TNF2368, 23 m, 15.5.2009, *AVE-L 366*. [6] **Vila Nova de Gaia.** Valadares — in an area with scanty pines, 29TNF2949, 24 m, 15.5.2009, *AVE-L 359*. **Beira Litoral:** [7] **Ovar.** Cortegaça, Dunas de Ovar, 29TNF2932, 8 m, 25.6.2009, *AVE-L 375, LEB-Lichenes 7813*. [8] **Aveiro.** S. Jacinto, Dunas de S. Jacinto (Reserva Natural das Dunas de S. Jacinto), 29TNF2302, 2 m, 23.4.2009, *AVE-L 349, LEB-Lichenes 7814*. [9] **Ílhavo.** Gafanha do carmo, Dunas da Gafanha, 29TNE2394, 13 m, 9.9.2009, *AVE-L 377, LEB-Lichenes 7815*. [10] **Vagos.** Gafanha do Areão, Dunas de Vagos, 29TNE2185, 21 m, 23.3.2009, *AVE-L 340*. [11] **Mira.** Seixo, Dunas de Mira, 29TNE2280, 24 m, 26.3.2009, *AVE-L 341, LEB-Lichenes 7816*. **Figueira da Foz.** [12] Quiaios, Dunas de Quiaios, 29TNE1654, 49 m, 4.9.2007, *AVE-L 383*; id., 29TNE1554, 49 m, 11.1.2007, *BG-L 88214, LEB-Lichenes 7817*; id., epiphytic on trunk of *P. pinea*, 29TNE1353, 45 m, 26.6.2009, *AVE-L 373*. [13] Serra da Boa Viagem, Mata do Prazo de Santa Marinha/Serra da Boa Viagem — in a pine forest in a mountainous area, 29TNE1149, 205 m, 26.6.2009, *AVE-L 371*; id., epiphytic on trunk of *P. pinea*, 29TNE1149, 200 m, 26.6.2009, *AVE-L 372*. [14] Costa de Lavos, Dunas da Leirosa, 29TNE1137, 21 m, 25.4.2009, *AVE-L 356, LEB-Lichenes 7818;* id., epiphytic on trunk of *P. pinea*, 29TNE1037, 16 m, 25.4.2009, *AVE-L 354, LEB-Lichenes 7819*. [15] Leirosa, Mata do Urso, 29TNE1132, 28 m, 25.4.2009, *AVE-L 352, LEB-Lichenes 7820*. [16] **Leiria.** Pedrógão, Mata do Pedrógão, 29SNE0416, 24 m, 25.4.2009, *AVE-L 344, LEB-Lichenes 7821*. **Estremadura:** [17] **Marinha Grande.** S. Pedro de Muel, Mata de Leiria, 29SNE0004, 49 m, 25.4.2009, *AVE-L 351*. [18] **Sintra.** Serra de Sintra, Ulgueira, Perímetro Florestal da Serra de Sintra (Parque Natural de Sintra Cascais) — in a pine forest in a mountainous area 29SMC5993, 260 m, 27.4.2009, *AVE-L 347*. **Baixo Alentejo:** [19] **Alcácer do Sal.** Comporta, Praia da Comporta, 29SNC1748, 24 m, 1.6.2009, *AVE-L 367*. [20] **Santiago do Cacém.** Relvas Verdes, Área Florestal de Sines, 29SNC2106, 75 m, 3.4.2009, *AVE-L 336*. **Algarve:** [21] **Aljezur.** Rogil, Praia de Vale dos Homens (Parque Natural do Sudoeste Alentejano e Costa Vicentina), 29SNB1537, 49 m, 30.5.2009, *AVE-L 370*. [22] **Vila do Bispo.** Sagres, Pinhal de Vale Santo (Parque Natural do Sudoeste Alentejano e Costa Vicentina) — epiphytic on branch of *P. pinaster*, 29SNB0300, 74 m, 31.5.2009 *AVE-L 369*.

Remarks — Recently separated as the sorediate counterpart of *C. chrysophthalma* (Smith et al. 2009, Tønsberg 1994), *C. flavovirens* may be confused with *C. candelaris*, which also occurs on pine trees in the study areas, but developing a more yellowish thallus with calycin (Elix & Kantvilas 2007, Laundon 1981). The chemical constitution of *C. flavovirens* (Elix & Tønsberg 2004, Laundon 1981, Tønsberg 1992) is variable, as Elix & Kantvilas (2007) refer to the presence of barbatic and conrhizocarpic acids, as well as epanorin, and the absence of atranorin in samples from the United Kingdom and Sweden.

Lepraria elobata Tønsberg

This species develops a diffuse, leprose, predominantly non-lobed and non-stratified, bluish-grey thallus, without medulla and with profuse more or less continuous fine soredia (Saag et al. 2009, Tønsberg 1992). Our sterile material of *L. elobata* contained atranorin, zeorin and stictic acid with satellites. Detailed descriptions are provided by Saag et al. (2009), Smith et al. (2009) and Tønsberg (1992).

Habitat and Distribution: Only one specimen of *L. elobata* was found epiphytic on *P. pinaster* bark at Dunas de Quiaios (Fig. 1A&B: 12), at an altitude of about 49 m, where it appears to be uncommon. This species occurs preferentiality on bark but is also known to grow on soil and siliceous rocks in shady and wet locations (Kukwa 2006). Regarding bark preferences, *L. elobata* seems to prefer deciduous to conifer trees (Saag 2007) and should be expected to occur in other habitats in Portugal.

Lepraria elobata is known from several European countries (Bayerová & Kukwa 2004, Boom et al. 1996, Coppins 2002, Czyżewska & Kukwa 2005, Diederich et al. 2009, Ekman & Tønsberg 2002, Feuerer 2009, Lukošienė & Naujalis 2009, Nimis & Martellos 2008, Osyczka & Stolarczyk 2005, Paz-Bermúdez et al. 2000, Prügger 2000, Tønsberg 1992) and North America (Saag et al. 2009, Tønsberg 2004).

Specimen examined - **PORTUGAL. Beira Litoral:** [Fig. 1A&B: 12] **Figueira da Foz.** Quiaios, Dunas de Quiaios, 29TNE1554, 49 m, 11.1.2007, *AVE-L 390, BG-L 88184.*

Remarks — *Lepraria nylanderiana* Kümmerl. & Leuckert is the most frequent *Lepraria* species at Dunas de Quiaios and is thought to be widespread in pine forests on sand dunes in the central west coast of Portugal. Both *L. elobata* and *L. nylanderiana* have a leprose, bluish-grey thallus, but *L. nylanderiana* has a delimited margin where minute lobes may appear in well-developed specimens. Also, *L. nylanderiana* usually has a whitish medulla, medium to coarse soredia, and thamnolic and roccellic acids and atranorin (Smith et al. 2009). *Lepraria elobata* is morphologically similar to *L. caesiella* R.C. Harris and *L. incana* (L.) Ach., which are differentiated chemically by the content in atranorin and zeorin, and divaricatic acid and zeorin, respectively (see Saag et al. 2009). Neither species has been found so far epiphytic on pine at Dunas de Quiaios.

Ochrolechia arborea (Kreyer) Almb. Fig. 1B

This species is characterized by a whitish grey, sorediate and thin thallus, which may be tuberculate in the centre. The soralia are mostly discrete and may be delimited and orbicular or more or less diffuse, rarely being confluent, but not covering the whole thallus (Boqueras et al. 1999, Tønsberg 1992). Our material was sterile and contained gyrophoric and lecanoric acids, lichexanthone (UV+ orange), and ± atranorin (Tønsberg 1992).

Habitat and Distribution — *Ochrolechia arborea* was found in localities along the western coast from Minho to Estremadura provinces (Fig. 1B, see also examined specimens) in Portugal's Atlantic and Mediterranean regions (ICN 2006). It was also found in mountainous locations at Serra da Boa Viagem (Figueira da Foz) (Fig. 1B, 13) and Serra de Sintra (Cascais) (Fig. 1B: 24),

having been recorded at altitudes up to 205 m at Serra da Boa Viagem. Most specimens were found on *P. pinaster*, but some were also found on *P. pinea*. Contrary to *C. flavovirens*, *O. arborea* is not restricted to maritime habitats and has been reported from inland countries such as Switzerland (Diederich & Sheidegger 1996) and Mongolia (Hauck & Javkhlan 2006). It is expected to occur in inland Portugal on a wide variety of phorophytes as reported in the literature (Boqueras et al. 1999, Christensen & Svane 2007, Kukwa 2009, Tønsberg 1992). The species has been noted as typical of shady habitats or soft barks (Johansson et al. 2007).

Ochrolechia arborea is currently known from several European countries (Andrianova et al. 2006, Boqueras et al. 1999, Brodo 1991, Christensen & Svane 2007, Coppins 2002, Diederich et al. 2009, Dietrich & Scheidegger 1996, Farkas et al. 2009, Feuerer 2009, Jabłońska & Kukwa 2007, Kukwa 2009, Liška et al. 2008, Nimis & Martellos 2008, Prügger 2000, Roux et al. 2008, Søchting & Alstrup 2008, Tibell 1992, Tønsberg 1992). It is also known from North and South America (Brodo 1991) and Asia (Hauck & Javkhlan 2006, John & Nimis 1998, Koneva 2007).

Specimens examined (square bracketed numbers refer to localities indicated in Fig. 1b): **PORTUGAL. Minho:** [1] **Caminha.** Moledo, Mata Nacional do Camarido, 29TNG1133, 15 m, 17.5.2009, *AVE-L 320, LEB-Lichenes 7822*. [2] **Viana do Castelo.** Chafé, Praia da Amorosa, 29TNG1510, 20 m, 17.5.2009 *AVE-L 323, LEB-Lichenes 7823*. **Beira Litoral:** [23] **Ovar.** Furadouro, 29TNF2823, 9 m, 23.3.2009 *AVE-L 310*. [11] **Mira.** Seixo, Dunas de Mira, 29TNE2280, 24 m, 26.3.2009, *LEB-Lichenes 7824*; id., on a branch of *Pinus pinaster*, 29TNE2280, 24 m, 26.3.2009, *AVE-L 312*. **Figueira da Foz.** [12] Quiaios, Dunas de Quiaios, on a branch of *P. pinaster*, 29TNE1353, 42 m, 14.4.2006, *AVE-L 389*; id., epiphytic on trunk of *P. pinea*, 29TNE1758, 49 m, 26.6.2009, *AVE-L 326, LEB-Lichenes 7825*. [13] Serra da Boa Viagem/ Mata do Prazo de Santa Marinha/Serra da Boa Viagem — in a pine forest in a mountainous area, 29TNE1149, 205 m, 26.6.2009, *AVE-L 328*; id., on trunk of *P. pinea*, 29TNE1149, 200 m, 26.6.2009, *AVE-L 324*. [16] **Leiria.** Pedrógão, Mata do Pedrógão — on a branch of *P. pinaster*, 29SNE0416, 24 m, 25.4.2009, *AVE-L 315*. **Estremadura:** [24] **Cascais.** Serra de Sintra, Penha Longa, Perímetro Florestal da Penha Longa (Parque Natural de Sintra Cascais) — in a pine forest in a mountainous area, on a branch of *P. pinaster*, 29SMC6490, 149 m, 27.4.2009, *AVE-L 317*.

Remarks — In our study area, *O. arborea* specimens growing on branches frequently developed roundish and delimited soralia, while those on trunks often generated confluent soralia. Orsellinic acid, detected in Spanish specimens (Boqueras et al. 1999), was not found in our material. *Ochrolechia microstictoides* Räsänen is another very common species in pine forests on sand dune areas in the centre of Portugal. This species, which has confluent soralia towards the centre of the thallus that give it a leprose appearance, contains variolaric and lichesterinic acids (Tønsberg 1992).

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Lichenological notes 2: *Lichenothelia convexa*, a poorly known rock-inhabiting and lichenicolous fungus

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ABSTRACT — *Lichenothelia convexa*, a saxicolous and lichenicolous microfungus, is reported new for the Czech Republic. The poorly known species is provided with a description and is illustrated.

KEY WORDS — *Lichenostigma saxicola, Lichenothelia paradoxa*

Introduction

Rock-inhabiting fungi (known as RIF) are common throughout the world. They are rich in diversity (Ruibal et al. 2009) and often collected by lichenologists who study saxicolous lichenized fungi. *Lichenothelia* D. Hawksw. is a cosmopolitan genus of RIF currently comprising 24 species (Robert et al. 2005). The genus *Lichenothelia* was introduced by Hawksworth (1981) for the *Microthelia aterrima* group of fungi with apothecioid ascomata with bitunicate asci, pseudoparaphyses, 1–3 septate to submuriform brown halonate ascospores, and a black areolate non-lichenized thallus. The generic type is *L. scopularia* (Nyl.) D. Hawksw., a widespread species on acid rock or mica schists in Europe and North America, which has mainly 3-septate to submuriform ascospores and an I– hymenium. Hawksworth included a second species in the genus, *L. metzleri* (J. Lahm) D. Hawksw., which has a similar thallus, but differs in having predominately 1-septate ascospores, an amyloid reaction on the outer layers of the asci (I+ blue), and occurs on calcareous rock and serpentine. It is also known from Europe and North America (Hawksworth 1981).

Aino Henssen published 18 new species in the genus on calcareous or non-calcareous rock (Henssen 1987). Five of the species had apothecioid

ascomata with pseudoparaphyses. Henssen emended the genus to include taxa with ascomata with asci developing in cavities within a centrum of spherical cells, describing 13 more species. Henssen's species varied from dispersed to contiguous areoles, becoming lobulate or effigurate thalli in some species. Some produced superficial hyphae called stolons, which usually produce new ascomata. Many taxa produced globose macroconidia, often on stalks, or meristematic offshoots. The centrums were either amyloid or non-amyloid. Some taxa were described as facultatively lichenicolous or fungicolous. The paper excited curiosity among lichenologists, who occasionally collected the more conspicuous rock-inhabiting fungi. But it proved perplexing. It was without a key and only a short diagnosis was given for each species. Most species were not illustrated and the pictures of the thalli, though good for the time, were over-all dark and too small. A monograph was apparently planned but was never completed. Authentic specimens of the new species were rare in herbaria and most types were unavailable for loan as Henssen continued her studies. Few of the types of Henssen's species are accessible now, although Hessen will eventually deposit them in Helsinki (H). Most lichenologists lost interest and stopped collecting the fungi when they found they could not be identified.

In the 23 years since Henssen's paper, only 4 new species have been described. *Lichenothelia antarctica* Øvstedal (Øvstedal & Lewis Smith 2001) has apothecioid ascomata with pseudoparaphyses and large muriform ascospores. It appears to be endemic to Antarctica. *Lichenothelia renobalesiana* D. Hawksw. & V. Atienza (Atienza & Hawksworth 2008) also has apothecioid ascomata with pseudoparaphyses. It has no thallus and is lichenicolous on *Verrucaria* species on limestone in Europe and North America (Kocourková & Knudsen 2009). *Lichenothelia spiratispora* Etayo is similar to *L. renobalesiana* but has smaller ornamented and halonate 1-septate ascospores and is lichenicolous on *Acarospora sphaerospora* H. Magn. (Etayo 2010). *Lichenothelia uralensis* Zhurb. (Zhurbenko 2008) from the Russian Federation has a cellular centrum and dispersed areoles and is known only from the type.

Recent pioneering studies of rock-inhabiting fungi using molecular analysis are renewing interest among mycologists (Ruibal et al. 2009). Currently several species of *Lichenothelia* are being cultured and sequenced. The reconstruction of their phylogeny is anticipated.

We became interested in saxicolous microfungi while studying lichenicolous fungi. We recently described *Lichenostigma saxicola* K. Knudsen & Kocourk. from southern California (Knudsen & Kocourková 2010), a taxon Henssen would have described in *Lichenothelia*. The new species has a non-amyloid centrum of spherical cells, 1-septate ascospores, dispersed thallus, produces stolons, and is both saxicolous and lichenicolous. From the Czech Republic

we examined two taxa with amyloid centrums with spherical cells, both facultatively lichenicolous. The more common species was eventually identified as *Lichenothelia convexa*. In this paper we publish a revised description of *L. convexa* and report it new for the Czech Republic. The other taxon may be new to science or else represent wider variation in *L. convexa* but needs further study and more specimens.

Materials & methods

Specimens of *Lichenothelia calcarea* Henssen, *L. convexa, L. metzleri, L. paradoxa* Henssen, *L. renobalesiana, L. scopularia, L. tenuissima* Henssen, as well as *Lichenostigma saxicola* and several probably undescribed taxa from Australia, Europe, North and South America were studied from B, FH, IMS, MIC, SBBG, PRM, UCR, UPS, and the Hb. Mycologicum J. Kocourková & K. Knudsen. Specimens have been examined using standard microscopical techniques with an Olympus SZX 7 Stereomicroscope or an Olympus BX 51 fitted with Nomarski differential interference contrast. Hand-made sections were studied in water and 10% KOH [K]. Amyloid reactions were tested in Lugol's iodine 1% [I] with and without pretreatment with K. Ascospore measurements were made in water with an accuracy of 0.5 µm and given in the form "(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)" and followed by the number of measurements (n); the length/breadth ratio of ascospore is indicated as l/b and given in the same way. Over-mature ascospores were few and excluded.

Macro- and microphotographs were taken with a digital Olympus D72 camera mounted on an Olympus SZX 7 Stereomicroscope and or an Olympus BX 51 fitted with Nomarski differential interference contrast.

Taxonomy

Lichenothelia convexa Henssen, Bibl. Lichenol. 25: 259 (1987). PLATE 1

TYPE: **GERMANY.** Hessen. Kr. Marburg, Wollenberg, on quartzite rock, 1980, Henssen 26750a (MB, **holotype**)

Vegetative hyphae, 4–5 µm in diam., brown, with granular surface, thick-walled, cells 7–10 µm long, branching not observed, immersed in substrate, usually one per stroma, often absent or hard to observe, I–. Thallus dispersed areolate, stromata sometimes congregated, infrequently confluent but separated by wide cracks, superficial on rock and lichens, not connected to other ascomata by networks of superficial hyphae. Stromata black, irregularly rounded, convex to somewhat flattened, mostly 100–200 × 90–120(–150) µm, some becoming as wide as 400 µm across, infrequently producing 1 to 3 superficial hyphae, "stolons" sensu Henssen (1987), black in dissecting microscope, black-brown with granular surface in light microscope, up to 1 mm long, but often shorter, one cell wide, cells various size, many 4–6 × 4–6 µm, often forming new stroma at tips of stolon, sometimes 3 stromata appearing linked in row. Stromata in

section pseudoparenchymatous, with round to angular cells mostly 4–5 µm in diam., external cells brown with granular surface, internal cells hyaline to light brown, I–, often sterile. Non-ostiolate, opening in apex by wall decay. Asci immersed in cavities in the stromata, bitunicate, broadly saccate to clavate, 20–30(–35) × 10–20 µm, fragile, mostly 8-spored. Intercellular spaces with an I+ blue, K/I+ blue gel, sometimes weak, reaction not observed when ascomata sterile. Ascospores hyaline to light brown when young, becoming darker brown, ellipsoid to broadly ellipsoid, faintly ornamented, halonate at least when young, cells not equal in size, 1–4 septate, sometimes becoming submuriform, (10.0–)10.5–*10.8*–11.5(–12.0) × (5.0–)6.0–*6.1*–6.5 µm (n = 20); l/b = (1.5–)1.7–*1.8*–1.9(–2.1). A few larger over-mature submuriform ascospores were seen, 14 × 6–6.5 µm, but were excluded. (Henssen 1987 gives ascospore measurements of 11–14 × 5.5–6.5 µm). Macroconidia globose, often absent, 10–15(–20) µm. Meristematic outgrowths also produced from surface of stromata of various sizes and shapes.

Ecology and substrate - On non-calcareous rocks and on saxicolous lichens. It is not host specific. In some specimens no damage to the host was observed. In a specimen from Krkonoše National Park, *Knudsen 12560, Lichenothelia convexa* is clearly pathological on the thallus of a *Lecidea* species.

Distribution. — Europe (Czech Republic, Germany, Sweden, United Kingdom), Asia (Turkey), North America in the United States (California, Colorado, Washington) (Halıcı et al. 2006; Hawksworth 1992; Henssen 1987).

Specimens examined. — **CZECH REPUBLIC.** East Bohemia. Krkonoše National Park, Krkonoše Mts, map of Czech Republic. 50°43'20"N 15°42'03"E, 1426 m, on granite outcrop and *Lecidea* species, 10 Oct. 2010, *K. Knudsen 12560 w/ J. Kocourková* (Hb. Mycologicum Kocourková & Knudsen). Central Bohemia. Distr. Praha, Pitkovice, Pitkovická stráň, 50°1'26"N 14°34'21"E, 276 m, on shale and *Rinodina aspersa*, 21 Sept. 2010, *J. Kocourková 7744* (Hb. Mycologicum Kocourková & Knudsen), on shale and *Acarospora gallica*, *Knudsen 12452* (BM, GZU, Hb. Mycologicum Kocourková & Knudsen); Nová Ves, Prokopské valley, Hemrovy rocks, 50°2'34.88"N 14°21'11.613"E, 265 m, on west-facing slope above road, on diabase and *Acarospora fuscata*, 7 June, 2010, *J. Kocourková 7404* (Hb. Mycologicum Kocourková & Knudsen); **GERMANY.** Hessen. Kreis Marburg/Biedenkopf, TK 25: 5117 Buchenau, Wollenberg, Wichtelhäuser, Hohler Stein, 385 m, on quartzite, 13 Aug. 1989, *H. T. Lumbsch & E. Mietzsch 6948* (B, topotype); Harz. Selketal, Meiseberg, 3 June 1990, *H. T. Lumbsch* (B).

Discussion

Lichenothelia convexa is widespread and probably under-collected. We report it as new for the Czech Republic, where it appears to be infrequent on non-calcareous rock (although often locally common on diabase and shale) and is often sterile. Unlike *L. scopularia*, *L. metzleri*, or *L. paradoxa*, all of which have conspicuous black areolate thalli, the dispersed areolate thallus of

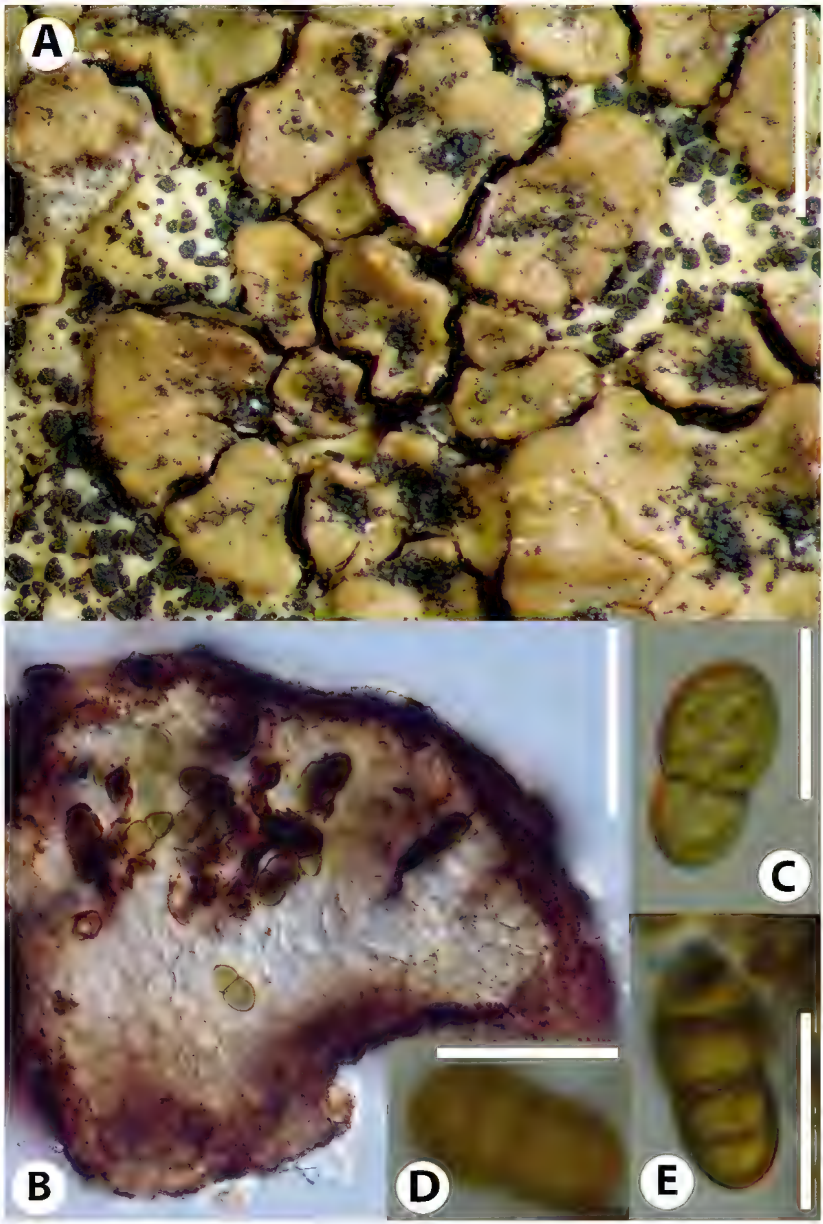

PLATE 1. A, *Lichenothelia convexa* on *Acarospora fuscata* and rock (*J. Kocourková 7404*, scale = 1 mm). B, Section of stroma of *L. convexa* (scale = 20 µm). C, Young 1-septate halonate ascospore (scale = 10 µm). D, Faintly ornamented surface of ascospore (scale = 10 µm). E, Spore with 3 septa becoming submuriform (scale = 10 µm).

L. convexa looks like the dispersed thalli of many rock-inhabiting microfungi seen on calcareous and non-calcareous rock throughout the world. Rarely does *L. convexa* form a more confluent thallus of stromata separated by wide, deep cracks.

In southern California, *Lichenothelia convexa* resembles *Lichenostigma saxicola,* which is distinguished by thinner usually hyaline or light brown stolons, 1-septate ascospores, and a non-amyloid centrum and which occurs at relatively low elevations in desert and coastal xeric habitats (Knudsen & Kocourková 2010). *Lichenothelia convexa* was collected in California at a mesic low elevation site (Silverwood, San Diego County) and higher elevation montane sites (Yosemite in the Sierra Nevada Mountains and Mt. Baldy in San Gabriel Mountains) (Henssen 1987). *Lichenostigma saxicola,* which has not been reported yet from Europe, is currently considered endemic to western North America and may be a predominately desert species.

Henssen (1987) originally described *Lichenothelia convexa* as having 3–4 septate ascospores, but our observations show the ascospores to be variably 1–4 septate. We also observed a K/I+ blue stain in the apex of the ocular chamber and lacking an amyloid ring. Further study of fresh material, best for taxonomic study, is needed, as good asci for staining were insufficient in our specimens. Sterile stromata are common.

Lichenothelia paradoxa is currently known only from Slovakia on quartzite but is expected in the Czech Republic. It resembles *L. scopularia,* but *L. paradoxa* has paraplectenchymatous stromata with asci forming in cavities rather than apothecioid ascomata with pseudoparaphyses. Otherwise the brown ascospores with 2 or 3 septa are similar in size to the ascospores of *L. convexa.*

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Mortierellomycotina subphyl. nov., based on multi-gene genealogies

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Abstract The *Mucoromycotina* unifies two heterogenous orders of the sporangiferous, soil-inhabiting fungi. The *Mucorales* comprise saprobic, occasionally facultatively mycoparasitic, taxa bearing a columella, whereas the *Mortierellales* encompass mainly saprobic fungi lacking a columella. Multi-locus phylogenetic analyses based on eight nuclear genes encoding 18S and 28S rRNA, actin, alpha and beta tubulin, translation elongation factor 1alpha, and RNA polymerase II subunits 1 and 2 provide strong support for separation of the *Mortierellales* from the *Mucoromycotina*. The existence of a columella is shown to serve as a synapomorphic morphological trait unique to *Mucorales*, supporting the taxonomic separation of the acolumellate *Mortierellales* from the columellate *Mucoromycotina*. Furthermore, irregular hyphal septation and development of subbasally vesiculate sporangiophores bearing single terminal sporangia strongly correlate with the phylogenetic delimitation of *Mortierellales*, supporting a new subphylum, *Mortierellomycotina*.

Key words — *Zygomycetes*, SSU rDNA, LSU rDNA, protein-coding genes, monophyly

Introduction

The type species of *Mortierella* Coem. 1863, *M. polycephala* Coem. 1863, was originally isolated from a parasitic interaction with a mushroom and named in honour of M. Du Mortier, the president of the Société de Botanique de Belgique (Coemans 1863). However, the common habit of mortierellalean species is as soil saprobes, enabling the fungi to grow on excrements, decaying plants, or (not infrequently) on decaying mushrooms and mucoralean fungi (Fischer 1892). Members of the *Mortierellales* do occasionally occur as opportunistic pathogens in animals and humans as well (de Hoog et al. 2009: 79). Because of the morphological differences to other mucoralean-like fungi, the genus was classified in a separate family, *Mortierellaceae* A. Fisch. 1892 (Fischer 1892), which is the only family within the *Mortierellales* Caval.-Sm. 1998 (Cavalier-

Smith 1998). In addition to *Mortierella,* the *Mortierellaceae* includes a further five genera, *Aquamortierella* Embree & Indoh 1967, *Dissophora* Thaxt. 1914, *Gamsiella* (R.K. Benj.) Benny & M. Blackw. 2004, *Lobosporangium* M. Blackw. & Benny 2004 (≡ *Echinosporangium* Malloch 1967, nom. illegit.), and *Modicella* Kanouse 1936. Currently, the *Mortierellales* are classified within the subphylum *Mucoromycotina* Benny 2007 with the *Mucorales* Fr. 1832 and *Endogonales* Moreau ex R.K. Benj. 1979 (Hibbett et al. 2007). This classification, based upon informal supertrees, abandons the phylum *Zygomycota* and recognizes four subphyla of uncertain phylogenetic position: *Zoopagomycotina, Entomophthoromycotina, Kickxellomycotina,* and *Mucoromycotina.* Supermatrix-based phylogenetic approaches using multiple genes in concatenated analyses place the *Mortierellales* basal to the *Mucorales* (Tanabe et al. 2004, James et al. 2006). In contradiction to this, the *Mortierellales* has also been shown to be more closely related to the *Dikarya* and *Glomeromycota* (Benny et al. 2001, Liu et al. 2009, Voigt et al. 2009).

This study provides evidence for the missing sister relationship between both orders. All attempts to constrain the monophyly were statistically insignificant and rejected under the null hypothesis. Therefore, the possibility of a common ancestor for both orders is unlikely. Combined Bayesian inference, maximum likelihood, maximum parsimony and distance phylogenetic analyses imply clear separation of the *Mortierellales* and the *Mucorales.* Both orders appear as well-separated, monophyletic groups in single and combined analyses. Two synapomorphic morphological features are identified that reliably characterize, strongly support, and justify the introduction of a new subphylum, *Mortierellomycotina.*

Materials & methods

Sequence and Phylogenetic Analyses. Nucleotide sequences encoding the nuclear small (18S) and large (28S) ribosomal RNA (rDNA), actin (act), translation elongation factor 1 alpha (tef), RNA polymerase II largest subunit (rpb1), RNA polymerase II second largest subunit (rpb2), alpha- (atub) and beta-tubulin (btub) from twenty-seven fungal species were retrieved from GenBank (www.ncbi.nlm.nih.gov), aligned and subjected to single and combined phylogenetic analyses using Bayesian inference, maximum likelihood, maximum parsimony and distance algorithms. In addition, amino acid sequences were also obtained from the protein-coding genes and used for validation of the tree topologies. Furthermore, nucleic acid sequences available from genome projects were screened for genomic regions, which are orthologous to the phylogenetic marker genes selected in this study. Accession numbers of the sequences retrieved from GenBank as well as the loci from the genome projects (http://genome.jgi-psf.org/ and www.broadinstitute.org/) are indicated by the taxon labels in Fig. 1. Individual alignments of the ribosomal DNA sequences and the protein-coding sequences were performed using ClustalW implemented in BioEdit version 7.0.9.0 (Hall 1999). Considering the triplet-based coding of the protein sequences, these sequences

were re-translated into their nucleic acid sequences using RevTrans (Wernersson & Pedersen 2003, www.cbs.dtu.dk/services/RevTrans/). Ambiguously and divergently aligned blocks were removed from the alignments prior to phylogenetic analysis, which was finally conducted in individual and all possible gene combinations of concatenated analyses. Two phylogenetic trees, which are representative for these analyses, are shown in FIG. 1. The phylogram presented in FIG. 1A bases on a combined alignment of the nuclear ribosomal DNA consisting of a total of 2103 characters (1518 and 585 characters for 18S and 28S rDNA, respectively). The phylogram presented in FIG. 1B was computed based on an aligned supermatrix concatenating a total of 5382 characters of the protein-coding genes (807; 1515; 1890 and 1170 characters for ACT, RPB1, RPB2 and BTUB, respectively). For the trees shown in FIG. 1A and 1B, respectively, the combined nucleotide data sets of the ribosomal DNA and the protein-genes were subjected to MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). The Bayesian inference was initiated from a random starting tree. Two independent runs with four chains each were conducted for 2,500,000 generations with samples collected every 2500th generation. After discarding the first 25% of the generated trees (burn-in) the consensus tree was calculated using the halfcompat option. Posterior probabilities (in percent) are indicated at the nodes and represent node confidence values. Additional bootstrap analyses (Felsenstein 1985; 50% majority rule) with 100 and 1000 replicates of maximum likelihood (RaxML; Stamatakis 2006; Stamatakis et al. 2008) and neighbor-joining (Saitou & Nei 1987) searches, respectively, were used to calculate clade stability. The amoeboid protist genus *Nuclearia* was chosen as outgroup taxon because it appears to be the closest extant relative to the fungi (Liu et al. 2009). The probability of alternative phylogenetic tree topologies constraining the phylogenetic relationship of the *Mucorales* and *Mortierellales* unified within a common monophyletic clade was evaluated by several independent statistical tests, such as (i) the Kishino-Hasegawa test (Kishino & Hasegawa 1989), (ii) the Templeton test (Templeton 1983) and (iii) the winning-sites test (Prager & Wilson 1988), based on the evaluation of the number of steps necessary to find the most parsimonious tree as implemented within PAUP*v4.0b10 (Swofford 1998). Additionally, the number of trees found after reaching a stationary phase (stationarity) from the constraint topology in the Bayesian analyses were compared to the number of trees without particular topological constraints using PAUP*v4.0b10.

IDENTIFICATION OF SEQUENCE SIGNATURES. Exonic nucleotide and protein sequences of the genes encoding ACT, RPB1, and TEF were identified as suitable marker genes for short unique sequence regions that differentiate the *Mortierellales* from the *Mucorales*. The sequence fragments were subjected to BLAST (Altschul et al. 1990, 1997) at NCBI in order to exclude identical sequence regions within the order *Mucorales*. These discriminatory sequences were defined as signature sequences and are displayed in TABLE 1.

Results & discussion

Phylogenetic tree reconstructions

In 2007, Hibbett et al. proposed a re-classification of the *Fungi*, which is now widely accepted, abandoning the phylum *Zygomycota* which was reported as

TABLE 1. Signature sequences derived from sequences in GenBank of all available genera of the subphylum *Mortierellomycotina* (as of 1 March 2010). Signature sequences were identified based on aligned amino and nucleic acid data from genes encoding actin, TEF, and RPB1. As verified by BLAST searches at NCBI, the signature sequences unequivocally characterize the *Mortierellales* and are not present in any member of the order *Mucorales*.

	AMINO ACID SEQUENCE [5` 3`]	NUCLEIC ACID SEQUENCE [5` 3`]
ACTIN (ACT), CONSENSUS	YLM(K/R)IL(M/L)ER GYSF(N/S/Q)T(S/T)A	TACCTSATGARGATYCTSMTGGAGCGHGGYTACTCYTTCHMBACCWCBGCY
Dissophora decumbens AJ287155	YLMKILMERGYSFNTSA	TACCTCATGAAGATCCTCATGGAGCGTGGTTACTCCTTCAACACCTCCGCC
Gamsiella multidivaricata AJ287168	.....S...	.....A.....T...TCG.....T...
Lobosporangium transversale AJ287156	.....S...	.....T.....T...TC...T..T..T
Mortierella alpina AM411535	.....S...	.....T.....C.....TC.....G...
M. chlamydospora AJ287167	.....S...	.....TCT.....
M. indohii EU736237	.....S.T.	.....C.....TC....A.....
M. minutissima EU736238	...R.....S.T.	.....G...T.....TC....A.....
M. minutissima EU736239	.....S.T.	.....T.....TC....A.....
M. polycephala AJ287169	.....S.T.	.....C.....T...TC....A....T
M. verticillata AJ287170	.....S.T.	.....TC....A.....
M. wolfii AJ287171	.....L.....Q...	.....G.....T..GC.....C.....T...C.G.....G...
TRANSLATION ELONGATION FACTOR 1ALPHA (TEF), CONSENSUS	VKKVGYNPK(S/T/A)V (P/A)FVPIS	GTCAAGAAGGTCGGTTACAACCCCAAGDCYGTYSCYTTCGTCCCCATYTCY
D. decumbens AF157247	VKKVGYNPKSVPFVPIS	GTCAAGAAGGTCGGTTACAACCCCAAGTCCGTTCCCTTCGTCCCCATCTCC
G. multidivaricata AF157260	.....T.A.....	.....A.T...G.....T
L. transversale AF157248	.....A.A.....	.....G.T...G.....T..T
M. alpina EU736263	.....A.....	.....G.T.....
M. chlamydospora AF157259	.....A.A.....	.....G.T..CG.T.....
M. indohii EU736264	.....A.....	.....G.T.....T
M. minutissima EU736265	.....A.....	.....G.T.....T...
M. polycephala AF157261	.....A.....	.....T...G.T.....
M. verticillata AF157262	.....A.....	.....G.....T...
RNA POLYMERASE II LARGEST SUBUNIT (RPB1), CONSENSUS	GWEGFLPTPAILKPKPL	GGTTGGGARGGWTTCCTRCCWACYCCTGCWATYCTMAAGCCCAAGCCYCTS
M. elongata AB097422	GWEGFLPTPAILKPKPL	GGTTGGGAGGGATTCCTGCCAACCCCTGCAATCCTCAAGCCCAAGCCCCTG
M. verticillata DQ294595	.....	.....A..T.....A..T..T.....T..T..A.....T..C

paraphyletic in several previous phylogenetic studies (e.g. Gehrig et al. 1996, Tanabe et al. 2000). The hypothesis of a close relationship between *Mortierellales* and *Mucorales* (classified as *Mucoromycotina*) was recently rejected by Liu et al. (2009) and, moreover, the justification for a classification of the *Mortierellales* into a separate supra-ordinal taxon distinct from the *Mucoromycotina* was proposed by Voigt et al. (2009). In our single and combined phylogenetic analyses both orders appear as distinct clades (Fig. 1A–B). Although, the actual relationship between both clades is not resolved, none of the sequence analyses supported a sister-clade relationship between *Mucorales* (clade 'a') and *Mortierellales* (clade 'b'). Based on ribosomal DNA data, the *Mortierellales* are more closely related to the *Glomeromycota* and the *Dikarya* (*Ascomycota* + *Basidiomycota*), but less related to the *Mucorales* (Fig. 1A). This relationship was also shown by Liu et al. (2009), but here the *Glomeromycota* were not included, using instead a phylogeny inferred from mitochondrially encoded sequences. Analyses of the protein-coding sequences show a minimal sister group relationship between *Mucorales* and *Glomeromycota* but fairly unresolved relationships between all other groups, probably due to a lack of informative characters and the phylogenetic signal of the marker genes for clade 'b' (Fig. 1B). Both phylogenies support the monophyly and also, therefore, the phylogenetic distinctiveness, of both clades 'a' and 'b' by bootstrap proportions (BP) of 100%. Eight-locus phylogenies using RaxML analyses resulted in the coherence of the *Mortierellales* supported by a BP of 77% grouping apart from the *Mucorales* (BP 90%; data not shown).

Rejecting the coherence of a *Mortierellales–Mucorales* clade

In single-locus analyses none of the eight loci supported the phylogenetic coherence of the *Mortierellales* with the *Mucorales* (Table 2). Using 18S, 28S rDNA and BTUB the individual clades of *Mortierellales* and *Mucorales* are supported by BP values of 100% each. But a coherent node for a unified *Mortierellales–Mucorales* clades collapses due to a BP below 50%. Also, no support of a monophyletic *Mortierellales–Mucorales* clade occurs if rpb1 and rpb2 are used, with 92% and 100% BP for a monophyletic *Mucorales* clade separated from the *Mortierellales*, respectively, for each individual gene. Since there is currently only one mortierellalean nucleotide sequence available for each of the phylogenetic markers RPB1 and RPB2, this order appears without statistical support. For act and tef only the *Mortierellales* are well supported (100% BP for TEF and 81% BP for ACT), there is also no statistical support for a monophyletic origin of both orders. With the exception of ATUB, from which no sequences were available for the *Mortierellales,* single-gene based bootstrapping of all other loci suggests the incoherence of the *Mucoromycotina* as a whole in its current circumscription, supporting the separation of the *Mortierellales* from the *Mucorales* (Table 2).

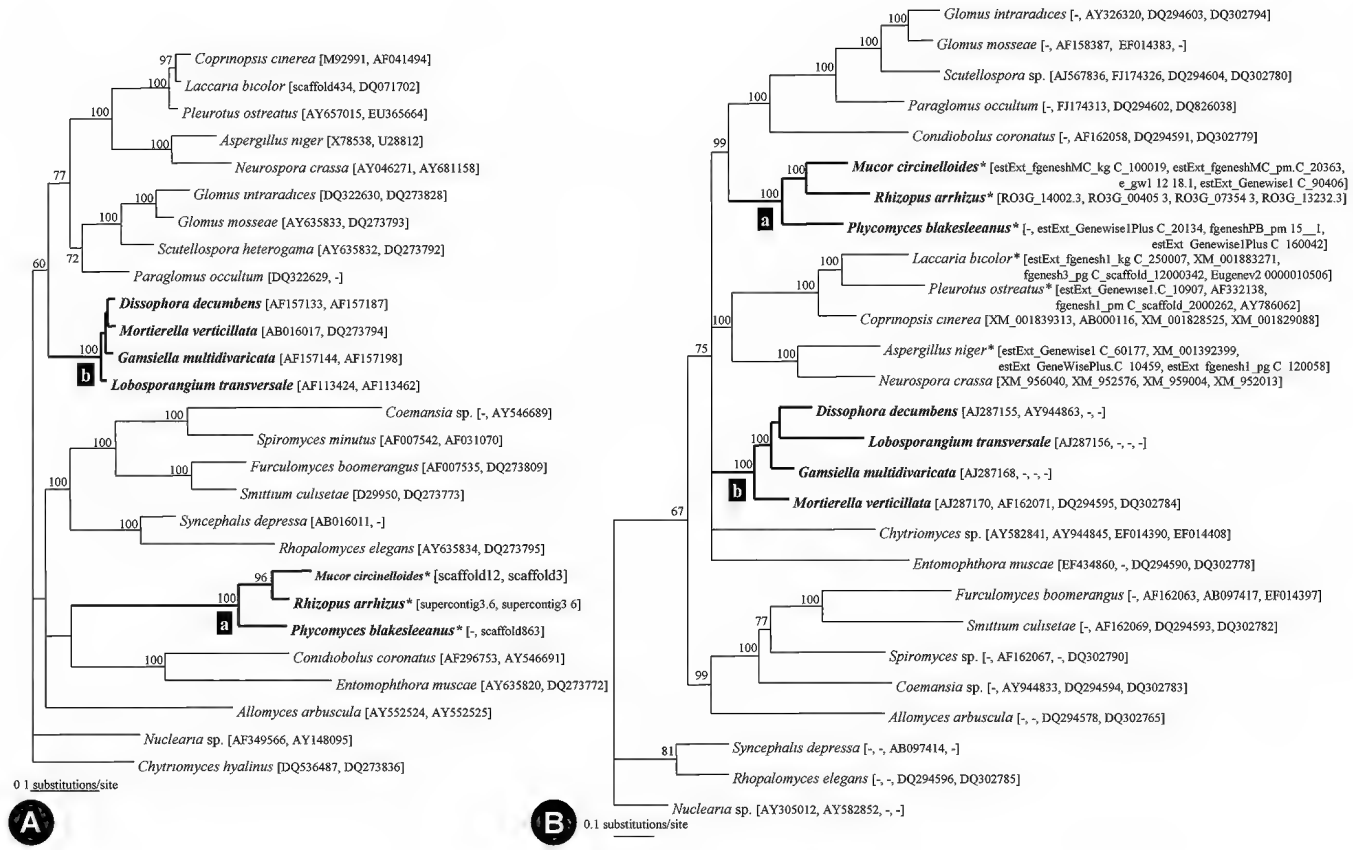
Coprinopsis cinerea [M92991, AF041494]
Laccaria bicolor [scaffold434, DQ071702]
Pleurotus ostreatus [AY657015, EU365664]
Aspergillus niger [X78538, U28812]
Neurospora crassa [AY046271, AY681158]
Glomus intraradices [DQ322630, DQ273828]
Glomus mosseae [AY635833, DQ273793]
Scutellospora heterogama [AY635832, DQ273792]
Paraglomus occultum [DQ322629, -]
Dissophora decumbens [AF157133, AF157187]
Mortierella verticillata [AB016017, DQ273794]
Gamsiella multidivaricata [AF157144, AF157198]
Lobosporangium transversale [AF113424, AF113462]
b
Coemansia sp. [-, AY546689]
Spiromyces minutus [AF007542, AF031070]
Furculomyces boomerangus [AF007535, DQ273809]
Smittium culisetae [D29950, DQ273773]
Syncephalis depressa [AB016011, -]
Rhopalomyces elegans [AY635834, DQ273795]
Mucor circinelloides* [scaffold12, scaffold3]
Rhizopus arrhizus* [supercontig3.6, supercontig3 6]
Phycomyces blakesleeanus* [-, scaffold863]
a
Conidiobolus coronatus [AF296753, AY546691]
Entomophthora muscae [AY635820, DQ273772]
Allomyces arbuscula [AY552524, AY552525]
Nuclearia sp. [AF349566, AY148095]
Chytriomyces hyalinus [DQ536487, DQ273836]
0 1 substitutions/site
A
Glomus intraradices [-, AY326320, DQ294603, DQ302794]
Glomus mosseae [-, AF158387, EF014383, -]
Scutellospora sp. [AJ567836, FJ174326, DQ294604, DQ302780]
Paraglomus occultum [-, FJ174313, DQ294602, DQ826038]
Conidiobolus coronatus [-, AF162058, DQ294591, DQ302779]
Mucor circinelloides* [estExt_fgeneshMC_kg C_100019, estExt_fgeneshMC_pm.C_20363, e_gw1 12 18.1, estExt_Genewise1 C_90406]
Rhizopus arrhizus* [RO3G_14002.3, RO3G_00405 3, RO3G_07354 3, RO3G_13232.3]
Phycomyces blakesleeanus* [-, estExt_Genewise1Plus C_20134, fgeneshPB_pm 15__1, estExt Genewise1Plus C 160042]
a
Laccaria bicolor* [estExt_fgenesh1_kg C_250007, XM_001883271, fgenesh3_pg C_scaffold_12000342, Eugenev2 0000010506]
Pleurotus ostreatus* [estExt_Genewise1.C_10907, AF332138, fgenesh1_pm C_scaffold_2000262, AY786062]
Coprinopsis cinerea [XM_001839313, AB000116, XM_001828525, XM_001829088]
Aspergillus niger* [estExt_Genewise1 C_60177, XM_001392399, estExt GeneWisePlus.C 10459, estExt fgenesh1_pg C 120058]
Neurospora crassa [XM_956040, XM_952576, XM_959004, XM_952013]
Dissophora decumbens [AJ287155, AY944863, -, -]
Lobosporangium transversale [AJ287156, -, -, -]
Gamsiella multidivaricata [AJ287168, -, -, -]
Mortierella verticillata [AJ287170, AF162071, DQ294595, DQ302784]
b
Chytriomyces sp. [AY582841, AY944845, EF014390, EF014408]
Entomophthora muscae [EF434860, -, DQ294590, DQ302778]
Furculomyces boomerangus [-, AF162063, AB097417, EF014397]
Smittium culisetae [-, AF162069, DQ294593, DQ302782]
Spiromyces sp. [-, AF162067, -, DQ302790]
Coemansia sp. [-, AY944833, DQ294594, DQ302783]
Allomyces arbuscula [-, -, DQ294578, DQ302765]
Syncephalis depressa [-, -, AB097414, -]
Rhopalomyces elegans [-, -, DQ294596, DQ302785]
Nuclearia sp. [AY305012, AY582852, -, -]
0.1 substitutions/site
B

TABLE 2. Bootstrap proportions of the *Mucorales* and *Mortierellales* nodes and the combined *Mucorales–Mortierellales* clade in single-locus analyses.

NA-sequences	18S	28S	ATUB	BTUB	RPB1	RPB2	TEF	ACT
Mucorales (Mu.)	100	100	78	100	92	100	51	54
Mortierellales (Mo.)	100	100	ni	100	nd	nd	100	81
Mu. + *Mo.*	0	0	-	0	0	0	0	0

*NA nucleic acid sequences coding for: 18S/28S: small/large subunit nuclear ribosomal DNA; ATUB/BTUB: alpha /beta tubulin; RPB1/2: RNA polymerase II subunit 1 or 2; TEF: translation elongation factor 1alpha; ACT: actin. ni: not included; nd: not determined because there is only one member of the *Mortierellales* included in the analysis.

Statistical tests for a monophyletic relationship between *Mortierellales* and *Mucorales*

The *Ascomycota, Basidiomycota* and *Kickxellomycotina* are well resolved and show high clade stability support in both analyses presented in FIG. 1. However, the relationships among those groups and the other groups are less clear-cut, showing no significant support values. Statistical tests for an alternative topology with a monophyletic *Mucorales–Mortierellales* clade allowed the exclusion of that hypothesis of a biordinal monophyletic relationship, which justifies their classification into a common subphylum. All tests rejected the phylogenetic alliance between *Mucorales* and *Mortierellales* with $P<0.0001$ under the assumption of the null hypothesis. In Bayesian inference analyses only 23 trees out of 2,002 were found after stationarity showing the topological constraint unifying both orders using the ribosomal DNA data set and no tree was found based on the combined protein data set. Therefore, it can be concluded that the probability of a monophyly for the *Mucorales–Mortierellales* clade is 1.15% for the ribosomal DNA data set and zero for the protein data set.

Identification of signature sequences

Based on ACT, TEF and RPB1, consensus amino and nucleic acid sequences were identified which represent diagnostic signatures unique for the *Mortierellales* (TABLE 1). These are the protein sequence YLM[K/R]IL[M/L]ERGYSF[[N/S/Q]T[S/T]A with its corresponding nucleic acid 5`-TAC CTS ATG ARG ATY CTS MTG GAG CGH GGY TAC TCY TTC HMB ACC WCB GCY-3` for act, VKKVGYNPK[S/T/A]V[P/A]FV PIS with 5`-GTC AAG AAG GTC GGT TAC AAC CCC AAG DCY GTY SCY

FIGURE 1. Bayesian inference analysis displaying the relationship between the orders *Mucorales* and *Mortierellales*. The phylogenetic reconstruction is based on A: a total of 2103 aligned nucleotide characters from the nuclear small and large subunit ribosomal DNA (1518 and 585 characters of the 18S and 28S rDNA, respectively) and B: a total of 5382 nucleotide characters from aligned protein-coding genes (807; 1515; 1890 and 1170 characters of ACT, RPB1, RPB2 and BTUB, respectively), each from 27 taxa. The protistan genus *Nuclearia* was chosen as outgroup taxon. Posterior probabilities are indicated as clade stability support values above the branches. Clade 'a' represents the subphylum *Mucoromycotina* and clade 'b' is represented by the *Mortierellomycotina*.

TTC GTC CCC ATY TCY-3` for TEF and GWEGFLPTPAILKPKPL with 5`-GGT TGG GAR GGW TTC CTR CCW ACY CCT GCW ATY CTM AAG CCC AAG CCY CTS-3` for RPB1 (TABLE 1). These signatures based upon sequences currently available in GenBank (as of 1st of March, 2010).

Identification of micromorphological traits

Typical micromorphological structures are shown in FIG. 2. The *Mucorales* develop a columella, a sterile swelling of the sporangiophore apex protruding into the multi-spored sporangium. Conversely, the *Mortierellales* lack this structure, suggesting that the columella is a synapomorphy of the *Mucoromycotina.* The vegetative hyphae of the *Mucorales* are coenocytic, with septation only rarely occurring below the columella within the sporangiophore. In contrast, the *Mortierellales* develop irregularly septate vegetative hyphae and, in addition, septa also appear among the hyphae lacking generative structures and sporangiophores. Also, the sporangiophores are typically subulate, being broad at the base and tapering towards the apex, whereas the sporangiophore diameters in the *Mucorales* remain more-or-less constant (Benny et al. 2001). Rhizoids are more pronounced and occur more frequently in the *Mucorales* than among the *Mortierellales;* giant cells are absent in the *Mortierellales.*

Taxonomic description

Mortierellomycotina Kerst. Hoffm., K. Voigt & P.M. Kirk, **subphylum nov.**

INDEX FUNGORUM IF 539369

Mycelium hypharum bifurcate ramosarum anastomosantium compositum. Sporangiophora basin inflata, apicem attenuata, erecta, non ramosa vel ramosa, ab initio non-septata, sed irregulariter septata ubi plene creta. Sporangia sphaerica, unispora vel multispora; columella carens. Sporangiosporae globosae usque ad ellipsoideas, vel irregulares, muris laevibus ornamentatisve. Stylosporae, rhizoides et chlamydosporae interdum praesentes. Zygosporae muris crassis; zygosporangium laeve; suspensores sine appendicibus.

TYPUS: *Mortierella* Coem. 1863

Zygomycota, incertae sedis. Mycelium with anastomosing hyphae, dichotomously branching, bearing stylospores. Hyphae sporangiferous, sporangiophores basally inflated and elongating towards the sporangiophore apex, erect, coenocytic initially, but irregularily septated at maturity. Asexual reproduction via sporangia and sporangiola. Sporangia sphaerical, multi-spored; columella absent. Ramifications gracilous, primarily horizontally expanding, erecting hyphae sometimes terminate with sporangiola. Spores globose to ellipsoid or irregular, smooth or ornamented. Rhizoids only occasional. Giant cells absent. Zygospores naked.

EXEMPLAR GENERA: *Mortierella, Dissophora, Modicella, Lobosporangium, Gamsiella, Aquamortierella.*

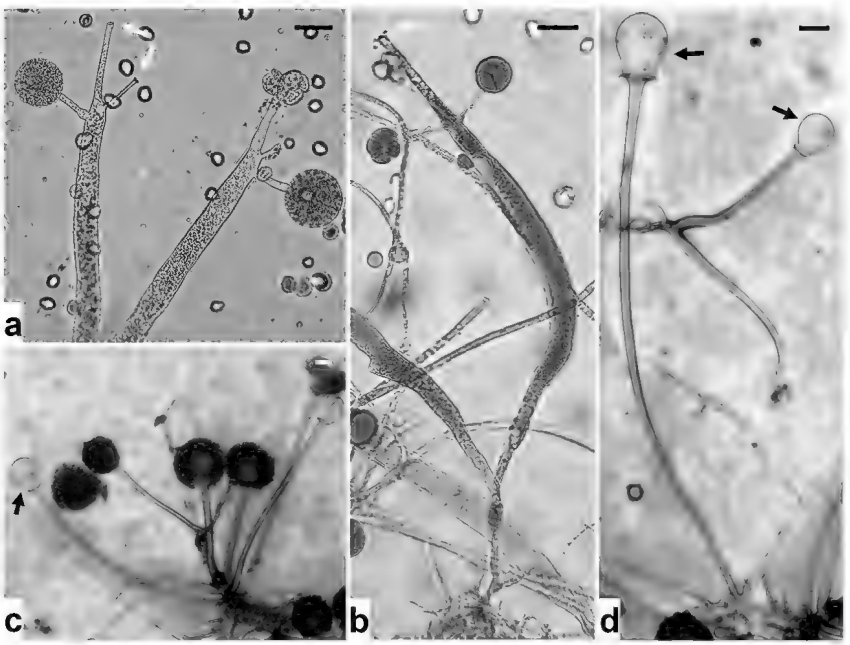

FIGURE 2. Micromorphological characteristics of prominent members of the subphyla *Mucoromycotina* and *Mortierellomycotina*. a) Sporangiophore of *Mortierella* sp. lacking a columella (white arrows). b) Tapering sporangiophore with rhizoids of *Mortierella* sp. Rejuvenating towards the sporangiophore apex is characteristic for the *Mortierellomycotina*. c+d) Non-tapering sporangiophores with rhizoids of *Rhizopus/Rhizomucor* sp. with sporangia. After spore release a well-developed columella remains (black arrows).

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The authors thank Martin Eckart for assistance in the establishment of a comprehensive sequence database comprising basal fungi and allied taxa. We also express our gratitude to John David (Royal Horticultural Society, Wisley, UK) for his help with the Latin diagnosis. Appreciation is extended to Dr. Hsiao-Man Ho (National Taipei University of Education, Taiwan), Sybren de Hoog (CBS-Knaw Fungal Diversity Centre, Utrecht, The Netherlands), Gerald L. Benny (University of Florida, Gainesville), and Dr. Shaun Pennycook for critical review of the manuscript and valuable remarks. This work was financially supported by the Deutsche Forschungsgemeinschaft (VO772/9-1).

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Three species excluded from *Melanopsamma* (*Ascomycetes*)

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Abstract *Melanopsamma* is a unitunicate ascomycete genus. However, examinations of the holotypes of *M. aggregata*, *M. merrillii*, and M. *pomiformis* var. *monosticha* has revealed that all were bitunicate. They are synonyms of *Astrosphaeriella africana*, *Didymosphaeria dimastospora*, and *Keissleriella sambucina*, respectively.

Key words morphology, mycology, taxonomy

Introduction

Melanopsamma Niessl ex Sacc. is a unitunicate ascomycete genus. As currently recognized, its main characters are superficial ascomata that are ostiolate, glabrous, and collabent when mature; reddish brown, 2- or 3-layered peridia; unitunicate, cylindrical, paraphysate, 8-spored asci; and ellipsoidal or fusiform, hyaline, 1-septate ascospores.

Of the approximately 110 *Melanopsamma* species names listed in Index Fungorum (2010), only 41 species are accepted in the genus (Kirk et al. 2008). To date, nine taxa have been transferred to other genera (Index Fungorum 2010), leaving about 80 names that are either synonyms or probably do not belong in the genus.

Most of the excluded species are bitunicate ascomycetes. The concept of bitunicate/unitunicate asci was not introduced until 1951 (Luttrell 1951). Before 1951 *Melanopsamma* included bitunicate taxa, which were otherwise very similar to unitunicate *Melanopsamma* species. The same situation occurred also with the unitunicate genus *Amphisphaeria* (Wang et al. 2004), in which earlier authors described many bitunicate taxa. There is no world monographic study on *Melanopsamma*.

In this paper, we exclude three bitunicate *Melanopsamma* species from the genus and reallocate them based on re-examination of their type specimens.

Materials & methods

Type specimens were borrowed from BPI, YAM and W. Dried material was rehydrated in water and fruiting structures were observed using a Zeiss 2000-C dissecting microscope. Slides of ascospores, asci, and sections of ascomata mounted in water were examined, photographed, and measured using a Zeiss Axioplan II image microscope.

Taxonomy

Astrosphaeriella africana D. Hawksw., Sydowia 38: 116.1986 ['1985']. FIGS 1–2.

Melanopsamma aggregata I. Hino & Katum., Bull. Fac. Agric. Yamaguti Univ. 6: 53.1955.

SPECIMEN EXAMINED: **Japan**, Sakaedani, Tokuyama, on dead stems of *Phyllostachys bambusoides* Siebold & Zucc. (*Poaceae*), 28 November 1954, I. Hino, YAM 20365, holotype of *Melanopsamma aggregata*.

Ascomata immersed, erumpent, arising singly in the substrate, 650–900 µm diam., hemispherical, black, base applanate, with a central papilla (FIG. 1). Asci bitunicate, 135–160 × 12–17 µm, 8-spored, cylindrical. Ascospores 50–57 × 5.5–6.5 µm, 2–3-seriate, fusiform, pale yellowish brown, 1-septate, constricted at the septum, with longitudinal wall striations (FIG. 2).

Didymosphaeria dimastospora Sousa da Câmara, Agron. lusit. 13: 123. 1951. FIGS 3–4.

Melanopsamma merrillii H.S. Yates, Philipp. Journ. Sc. 12: 376. 1917.

SPECIMEN EXAMINED: **Philippines**, Alabat Island, substrate undetermined, 21 December 1916, E.D. Merrill 10554, BPI 612151, holotype of *Melanopsamma merrillii*.

Ascomata bitunicate, 400–600 µm diam., globose, erumpent (FIG. 3). Ostiole raised, lateral. Pseudoparaphyses filaments 1–1.5 µm wide. Asci 100–135 × 9–12 µm. Ascospores ellipsoidal, grey, 17–22 × 6–9 µm, often widened at the septum, 1-septate, cells equal, without ornamentation, but sometimes with brownish gel at the poles (FIG. 4).

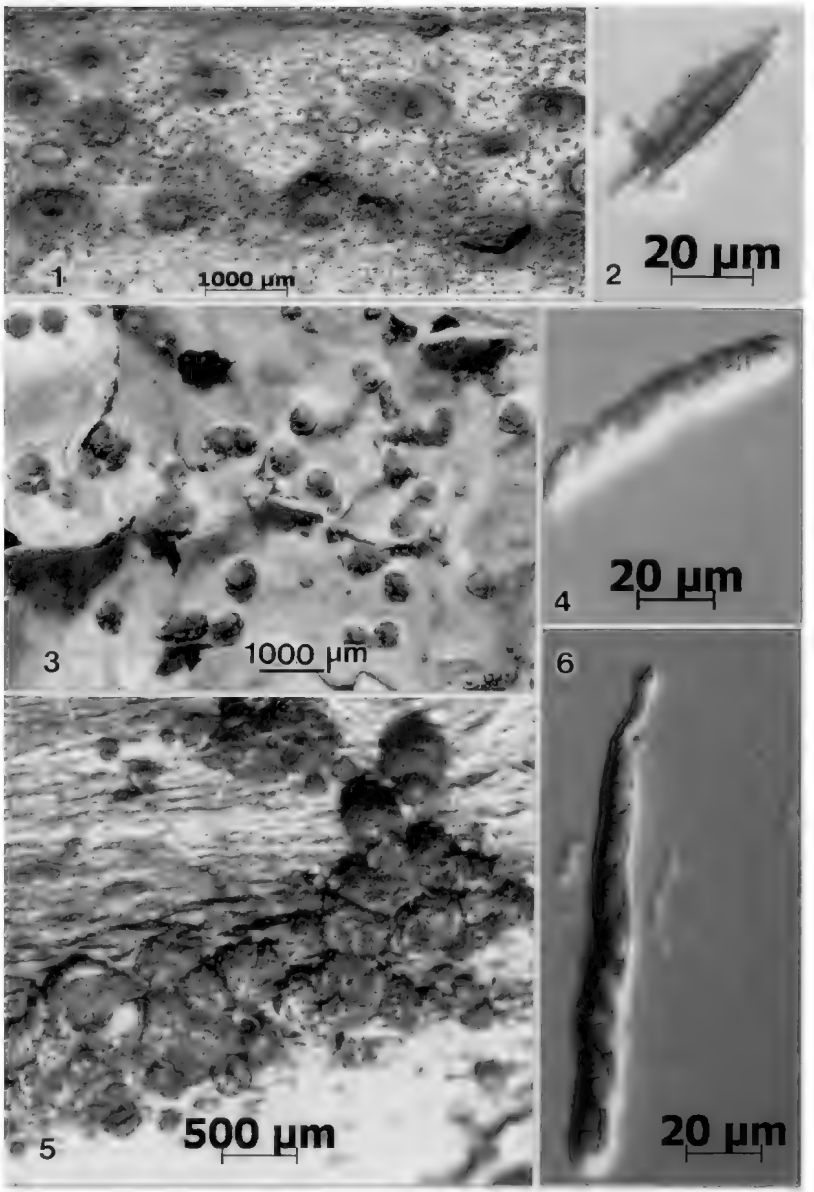
NOTES: *Didymosphaeria* Fuckel was monographed by Aptroot (1995), who accepted seven species out of 550 named taxa. The excluded taxa belong to other similar genera, which are bitunicate or unitunicate ascomycetes.

Keissleriella sambucina (Rehm) Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 128: 582. 1919. FIGS 5–6.

Melanopsamma pomiformis var. *monosticha* Keissl., Beih. Bot. Centrlbl., Abt. II, 29: 400. 1912.

SPECIMENS EXAMINED: **Slovenia**, Carniola, on dry branch of *Sorbus aria* (L.) Crantz (*Rosaceae*), July 1908, leg. & det. K.V. Keissler, W 1908/9129, holotype of *Melanopsamma pomiformis* var. *monosticha*. **Austria**, Ybbsitz, on *Cydonia japonica* Pers. (*Rosaceae*), March 1909, W 1939/0113, [as Melanopsamma *amphisphaeria* Schulzer & Sacc.]

Ascomata subglobose, semi-immersed to superficial, gregarious to crowded, black, 300–500 µm in diam., 300–450 µm in height (FIG. 5). Ostiole papillate



FIGURES 1–6. FIGS 1–2: *Astrosphaeriella africana* (from holotype of *Melanopsamma aggregata*): 1 – habit of ascomata on host surface; 2 – ascospores. FIGS 3–4. *Didymosphaeria dimastospora* (from holotype of *Melanopsamma merrillii*): 3 – habit of ascomata on host surface; 4 – intact ascus, ascospores. FIGS 5–6. *Keissleriella sambucina* (from holotype of *Melanopsamma pomiformis* var. *monosticha*): 5 – habit of ascomata on host surface; 6 – ascus, ascospores.

with short, blackish brown setae, not collabent. Pseudoparaphyses trabeculate. Asci bitunicate, cylindrical, 8-spored, 110–140 × 11–13 μm (Fig. 6). Ascospores 16–20 × 6–8 μm, hyaline, ellipsoidal, asymmetrical with upper hemispore wider and more obtuse than lower, 1-septate, wall smooth, constricted at the septum (Fig. 6).

Key to the genera

1a. Ascomata collabent when mature, asci unitunicate ..... *Melanopsamma*
1b. Ascomata not collabent, asci bitunicate 2
2a. Ascomata gregarious to crowded, ascospores ellipsoidal or fusiform, 1–multi-septate ..... *Keissleriella*
2b. Ascomata scattered 3
3a. Ascospores 1-seriate, ellipsoidal, 1-septate ..... *Didymosphaeria*
3b. Ascospores 2–3-seriate, elongate-fusiform, 1–5-septate ..... *Astrosphaeriella*

Acknowledgments

Drs Eric McKenzie and André Aptroot are deeply thanked for pre-submission reviews. The author would also like to thank the curator of herbarium BPI, W, YAM, for loan of specimens. Thanks are extended to Dr Shaun Pennycook for nomenclatural review. This work was supported by the Funds of the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-YW-Z-004).

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Tubakia seoraksanensis, a new species from Korea

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ABSTRACT An unknown species of *Tubakia* was collected recently from *Quercus mongolica* on Seoraksan Mountain, GangWon Province, in Korea. This species was characterized with cultural, ITS region sequence, and morphological data. After comparison with known species of *Tubakia*, this species is here described as *Tubakia seoraksanensis*, sp. nov.

KEY WORDS *Ascomycota, Diaporthales, Dicarpella,* forest pathogen, *Sordariomycetes*

Introduction

The anamorphic genus *Tubakia* B. Sutton (*Diaporthales, Ascomycota*) causes leaf spot diseases on *Castanea, Quercus,* and various other trees and shrubs primarily in the *Fagaceae* (Sinclair et al. 2005). Currently, five species are recognized in *Tubakia*, namely *T. castanopsidis* (T. Yokoy. & Tubaki) B. Sutton, *T. dryina* (Sacc.) B. Sutton, *T. japonica* (Sacc.) B. Sutton, *T. rubra* (T. Yokoy. & Tubaki) B. Sutton, and *T. subglobosa* (T. Yokoy. & Tubaki) B. Sutton (Belisario 1991, Limder & Cash 1945, Sutton 1973, Yokoyama & Tubaki 1971). *Tubakia* has been associated with the teleomorphic genus *Dicarpella* Syd. & P. Syd. with the single species *D. dryina* Belisario & M.E. Barr connected to *T. dryina* (Belisario 1991).

In Korea, three species of *Tubakia* have been reported: *T. japonica* on *Castanea crenata* Siebold & Zucc., *Quercus acutissima* Carruth., and *Q. aliena* Blume; *T. rubra* on *Q. serrata* Murray; and an undescribed *Tubakia* sp. on *Q. rubra* L. (Cho et al. 2004, Lee et al. 1991,). In August 2009, specimens of *Tubakia* were collected on leaves of *Quercus mongolica* Fisch. ex Ledeb. (Mongolian oak) in Seoraksan Mountain, Gangwon Province, Korea. *Quercus mongolica* is a mountain tree that grows on ridgelines in Korea and is resistant to drought and wind conditions (Lee 1997). We determined that the specimens represented a previously undescribed species of *Tubakia*, which we then

analyzed using cultural, molecular, and morphological methods and compared with known species. The new species is described below.

Materials & methods

Fungal Isolations

Diseased leaves of *Quercus mongolica* were collected from nature. Isolates from fructifications were made from two different areas on the leaves, namely necrotic areas surrounding veins and leaf spots. Cultures were grown on malt extract agar (MEA, 1.5% malt extract and 2.0% agar) at 25°C in the dark. Specimens were deposited at the U.S. National Fungus Collections, Beltsville, Maryland, USA (BPI 880798 and BPI 880799) and cultures were deposited in CBS Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS 127490, CBS 127491, CBS 127492, and CBS 127493).

Morphological examination

Observations of microscopic structures were made from material mounted in distilled water and measured using a compound light microscope (Axiophot, Zeiss, Germany). Colors of cultures were described using Munsell soil color charts (Macbeth 2000).

DNA extraction, PCR, sequencing, analysis of sequence data

DNA was isolated from cultures on MYEA (2% malt extract, 0.2% yeast extract, and 1.5% agar) grown for about 10 to 14 days at room temperature. Mycelium was used for DNA extraction using PrepMan™ Ultra reagent following the manufacturer's instructions (Applied Biosystems, Foster City, CA). The internal transcribed spacer regions 1 and 2 and the 5.8 S rDNA hereafter referred to as ITS were amplified using the primer pair, ITS-1F and ITS4 (Gardes & Bruns 1993). Amplification of the ITS region was achieved with initial denaturation at 85°C 2 min; 35 cycles, each consisting of denaturation at 94°C for 1 min 35 sec, annealing at 49°C for 1 min, and extension at 72°C for 1 min; and final extension at 72°C for 15 min. PCR products were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. Automated sequencing was performed at the Iowa State University DNA Sequencing and Synthesis Facility (Ames, IA). Reactions were set up using Applied Biosytems (Foster City, CA) Prism BigDye Terminator v3.1 cycle sequencing kit with AmpliTaq DNA polymerase, FS and analyzed on an Applied Biosystems 3730 DNA analyzer.

Sequences were deposited at GenBank sequence database (HM991734, HM991735, HM991736, and HM991737). A GenBank Blast search was performed with the resulting sequence data and a comparison was made to existing *Tubakia* ITS region sequences. All sequences of *Tubakia* species were aligned and compared using the jPHYDIT program (Jeon et al. 2005).

Results: Analysis of sequence data & taxonomy

The ITS sequences from each of the four isolates were identical. The GenBank Blast search revealed the closest identifiable match as *Dicarpella dryina* (= *Tubakia dryina*, accession no. AY853242, culture CBS 115970) with 94% maximum identity. Additional

comparison with sequences of all other *Tubakia* species revealed a close relationship with *Tubakia japonica* (unpublished data). In the ITS region alignment produced in PHYDIT, sequences of *Tubakia seoraksanensis* were 98% similar to *T. japonica* and 97.8% similar to *D. dryina* (accession no. AY853242) (unpublished data).

Tubakia seoraksanensis H.Y. Yun, **sp. nov.** FIG. 1

MYCOBANK MB 519212

Coloniis in MEA similis Tubakiae japonicae *coloratis, sed conidiis minoribus, 13-25 µm × 10-15 µm, differt.*

TYPE: Korea, Gangwon, Seoraksan National Park, Seorak-dong, Sokcho-si, Gangwon-do, on living leaves of *Quercus mongolica*, 31.VIII.2009, coll. Hye Young Yun. **Holotype**, BPI 880799 (cultures CBS *127490*, ITS GenBank HM991734; CBS *127491*, ITS GenBank HM991735); **Isotype**, BPI 880798 (cultures CBS *127492*, ITS GenBank HM991736; CBS *127493*, ITS GenBank HM991737).

ETYMOLOGY: referring to the type locality.

LEAF SPOTS epiphyllous, occasionally amphigenous, 2–10 mm diam., globose to broadly ellipsoidal on blade, at times necrotic areas ellipsoidal to fusiform, extending along midrib or veins, pale brown, margin regular or irregular in shape, dark brown; typically discrete, may become confluent. CONIDIOMATA epiphyllous, occasionally amphigenous, frequently but not always associated with spots, scattered to gregarious, sometimes confluent, especially near veins, superficial, easily removed from leaf surface, brown to dark brown, scutellate, each attached to leaf by a central columella, circular or somewhat irregularly subcircular as viewed from above, more or less plane as viewed from side. SCUTELLA 90–160 × 90–130 µm, membranous, composed of thick-walled, pale brown to brown, 4–6 µm diam., septate hyphae radiating, typically bifurcating up to three times from a central disc that consists of a single hyaline cell towards margin, acute and cornute at margin that is fringed and unattached to substrate. CONIDIOGENOUS CELLS formed on underside of scutella, radiating downward and towards margin, 14–22 × 3–5 µm, cylindrical or slightly clavate to fusiform, narrowing to a thin point at neck, pale brown to hyaline. CONIDIA 13–25 × 10–15 µm (Q = 1.15–1.56), acrogenous, blastic, subglobose, broadly ellipsoid to ellipsoid, hyaline, becoming pale yellowish brown, walls smooth, thickening in age, with a prominent frill at base. Microconidia unknown.

IN CULTURE: Colony (after 10 days on MEA at 25°C in the dark) 32–44 mm diam., low velutinous to fuzzy, wrinkled, margin with uneven growth, whitish to pale yellow (8/1–2), reverse wrinkled, center darker, becoming progressively paler towards margin, olive brown, light olive brown to yellow (4/3, 5/6, 8/6), margin white (8/1), without sporulation. Mycelium branching, septate, 3.2–4.8 µm diam., hyaline or slightly brownish in mass, some hyphae forming short coils on side branches.

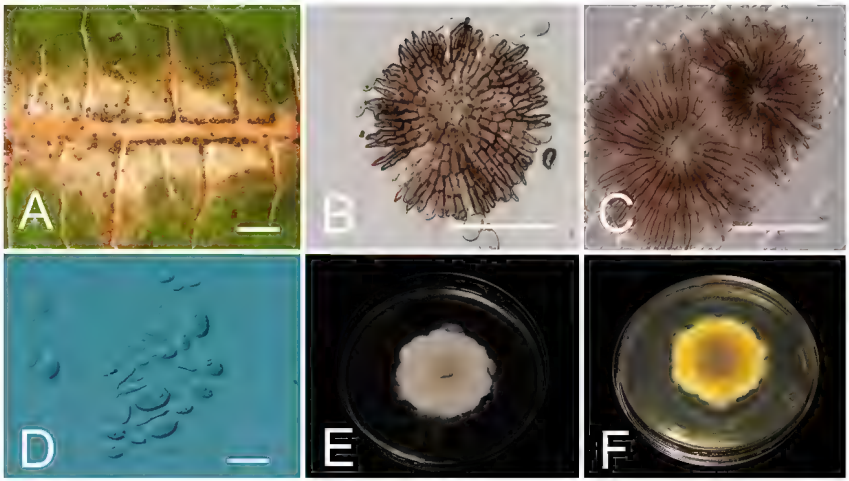

FIG. 1. *Tubakia seoraksanensis* (A–D, holotype; E–F, CBS 127491). A. Necrotic leaf spot and conidiomata along leaf midrib of *Quercus mongolica*. B. Scutellum and conidia by light microscopy (LM). C. Scutella and conidia by LM. D. Conidia and conidiophores by LM. E. Colony on MEA. F. Colony reverse on MEA. (Scale bars: A = 2mm, B–C = 50 µm, d = 20 µm)

HABITAT & DISTRIBUTION — Known only from *Quercus mongolica* at the type locality in Seoraksan National Park, Republic of Korea.

COMMENTS — *Tubakia seoraksanensis* can be distinguished from all known species of *Tubakia* except *T. japonica* by its larger conidia. In culture on MEA, the colony color of *T. seoraksanensis* resembles that of *T. japonica* but is readily distinguished from the other species as illustrated in plate 2 of Yokoyama & Tubaki (1971).

Conidial size is the primary morphological difference distinguishing *T. japonica* and *T. seoraksanensis,* with *T. japonica* producing conidia that are much larger (40–55 × 35–45 µm) compared to those of *T. seoraksanensis* (13–25 × 10–15 µm). Yokoyama & Tubaki (1971) noted that the sizes of scutella, columellae, and conidia and hosts are useful in differentiating species. As with Jones & Holcomb (1978), Glawe & Crane (1987), and Taylor (2001), we found columellar characters untenable due to difficulty in observing these fragile structures and unreliable historical data.

Isolates of *T. seoraksanensis* from the different parts of the leaves (e.g., leaf blades or surrounding veins) produced identical sequences and conidiomatal morphology. However, necrotic areas surrounding veins tended to extend some distance along veins, be more irregular in shape, and were associated with a greater number of epiphyllous and hypophyllous conidiomata. The leaf spots on the leaf blades tended to be more confined in size, more regularly circular

in shape, and associated with a smaller number of epiphyllous and especially hypophyllous conidiomata.

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Two new species of *Hymenochaete* (*Hymenochaetales*) from China

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ABSTRACT — Two new species, *Hymenochaete megaspora* and *Hymenochaete acerosa* are described from Xizang Autonomous Region (Tibet), China. *Hymenochaete megaspora* distinguishes from other species by its large basidiospores and heavily encrusted setae; while *Hymenochaete acerosa* is unique for its acerose setae, loosely interwoven hyphae and relatively large basidiospores.

KEY WORDS — *Hymenochaetaceae*, taxonomy, wood-inhabiting fungi

Introduction

Hymenochaete is one of the important genera in *Hymenochaetaceae*. This group of wood-decaying fungi has been studied by several authors in China, who recorded 39 taxa from the country (Dai et al. 2000, Xu et al. 2003, Zhang & Dai 2005, Dai & Niemelä 2006, Dai 2010, 2011, He 2010). Dai (2010a) revised the genus in China systematically and illustrated 23 species. However, in comparison with the total number of known species worldwide (ca. 110), the genus is still poorly known in China, and many species remain undiscovered.

Xizang Autonomous Region (Tibet) and its adjacent areas are largely unknown mycologically. Despite reports on polypores in the region by Dai et al. (2004, 2007a, b) and Yuan & Dai (2008), many species of other groups of wood-decaying fungi are still unrecorded. Recently, during a survey of wood-inhabiting fungi in Xizang Autonomous Region approximately 100 specimens of *Hymenochaete* were collected. Two species are distinctly different from known species of the genus. Therefore they are described as new species in this paper.

Materials & methods

Voucher specimens are deposited in the herbarium of Beijing Forestry University (BJFC), and the microscopic procedure follows Cui & Dai (2008). In the text the

following abbreviations are used: L = mean spore length (arithmetical average of all spores), W = mean spore width (arithmetical average of all spores), Q = variation in the L/W ratios between the specimens studied (quotient of the mean spore length and the mean spore width of each specimen), n = the number of spores measured from given number of specimens. In presenting the size range of spores, 5% of the measurements were excluded from each end of the range, and the measurements were given in parentheses. IKI stands for Melzer's reagent, KOH for 5% potassium hydroxide, and CB is the abbreviation of Cotton Blue. IKI– = inamyloid and nondextrinoid, CB– = acyanophilous. Special color terms follow Petersen (1996).

Taxonomy

Hymenochaete megaspora S.H. He & Hai J. Li, **sp. nov.** FIGS. 1–2

MycoBank MB 519354

Carpophorum annuum, effusum vel effuso-reflexum, margine leviter elevatum, laxe adnatum, coriaceum, in sicco durum fragileque, 150–600 µm crassum. Superficies pilei griseo-brunnnea vel atro-grisea, tomentosa, concentrice sulcata et zonata. Hymenophorum laeve vel tuberculatum, submurinum vel vinaceo-griseum. Cortex, tomentum et stratum hypharum adsunt; cortex ex hyphis agglutinatis compositus, 10–25 µm crassus. Systema hypharum monomiticum, hyphae generativae sine fibulis, hyalinae vel flavo-brunneae, tenuiter tunicatae vel crasse tunicatae, interdum septatae, rare ramosae, plus minusve intertextae, 2–4 µm diam; setae in hymeniis stratosis abundae, subulatae, acutae, parte superiore cum crystallis incrustatae, (80–)90–120(–140) × 8–13(–14) µm; cystidia desunt; hyphidia hyalina, 2–3 µm in diam; basidia clavata, 4 sterigmatibus praedita, 25–32 × 5–8 µm; sporae late ellipsoideae, IKI –, CB –, (7–)7.5–10(–11) × 5–7 µm.

Type: China. Xizang Autonomous Region, Linzhi County, Lulang, alt 3700 m, on dead branch of *Quercus* (*Fagaceae*), 17.IX.2010 He 328 (**Holotype,** BJFC).

Etymology: *megaspora*, refers to the large basidiospores.

Fruitbody: Annual, effused or effused-reflexed with slightly elevated margins, loosely adnate, easily detached, coriaceous without odour or taste when fresh, becoming hard and brittle when dry, 150–600 µm thick, resupinate part up to 7cm long, reflexed part short and broad, projecting 0.1–0.4 cm. Pileal surface grayish brown to dark gray, silky, tomentose, concentrically sulcate and zonate; margin thin, lighter than pileal surface. Hymenophore smooth or tuberculate, slightly irregularly cracked when old, pale mouse-gray to vinaceous gray; resupinate margin thinning out, indistinct, silky, slightly fimbriate, lighter than hymenophore, cinnamon to yellowish brown, up to 0.5 cm.

Hyphal structure: Hyphal system monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

Subiculum: Cortex, tomentum and hyphal layer present. Cortex composed of strongly agglutinated hyphae, 10–25 µm. Setal layer thickening in old specimens, sometimes a hyphal layer present between rows of setae. Generative hyphae hyaline to yellowish brown, thin- to distinctly thick-walled with a

FIG. 1. Fruitbody of *Hymenochaete megaspora* (He 302, paratype).

narrow lumen, occasionally septate, rarely branched, regularly arranged, more or less interwoven, 2–4 µm in diam.

STRATIFIED HYMENIUM: Hyphae in this layer similar to those in subiculum, yellowish to yellowish brown, thick-walled, more or less agglutinated, interwoven, 2–3.5 µm in diam. Setae abundant, subulate, dark brown, thick-walled with a wide or narrow lumen; tip acute, always encrusted with small crystals; setae projecting up to 80 µm above the hymenium, (80–)90–120(–140) × 8–13(–14) µm; cystidia absent; hyphidia present in sterile specimens, hyaline, slightly conical, 2–3 µm in diam; basidia clavate, with four sterigmata and a simple septum at base, 25–32 × 5–8 µm; basidioles in shape similar to basidia, but smaller.

SPORES: Basidiospores broadly ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (7–)7.5–10(–11) × 5–7 µm, L = 8.59 µm, W = 5.94 µm, Q= 1.40–1.50 (n = 60/2).

ADDITIONAL SPECIMENS (PARATYPES) EXAMINED: **CHINA. XIZANG AUTONOMOUS REGION**, Linzhi County, Lulang, alt 3700 m, on dead branch of *Quercus* (*Fagaceae*), 16.IX.2010 He 302; 18.IX.2010 He 334; Bomi County, on dead branch of *Quercus* (*Fagaceae*), 19.IX.2010 He 351 (Paratypes, BJFC).

REMARKS: *Hymenochaete megaspora* is characterized by large and broadly ellipsoid basidiospores, encrusted setae, and the presence of a cortex. Its basidiospores are similar to those of *H. gigaspora* D.A. Reid (6.5–8.5 × 5.2–6.5

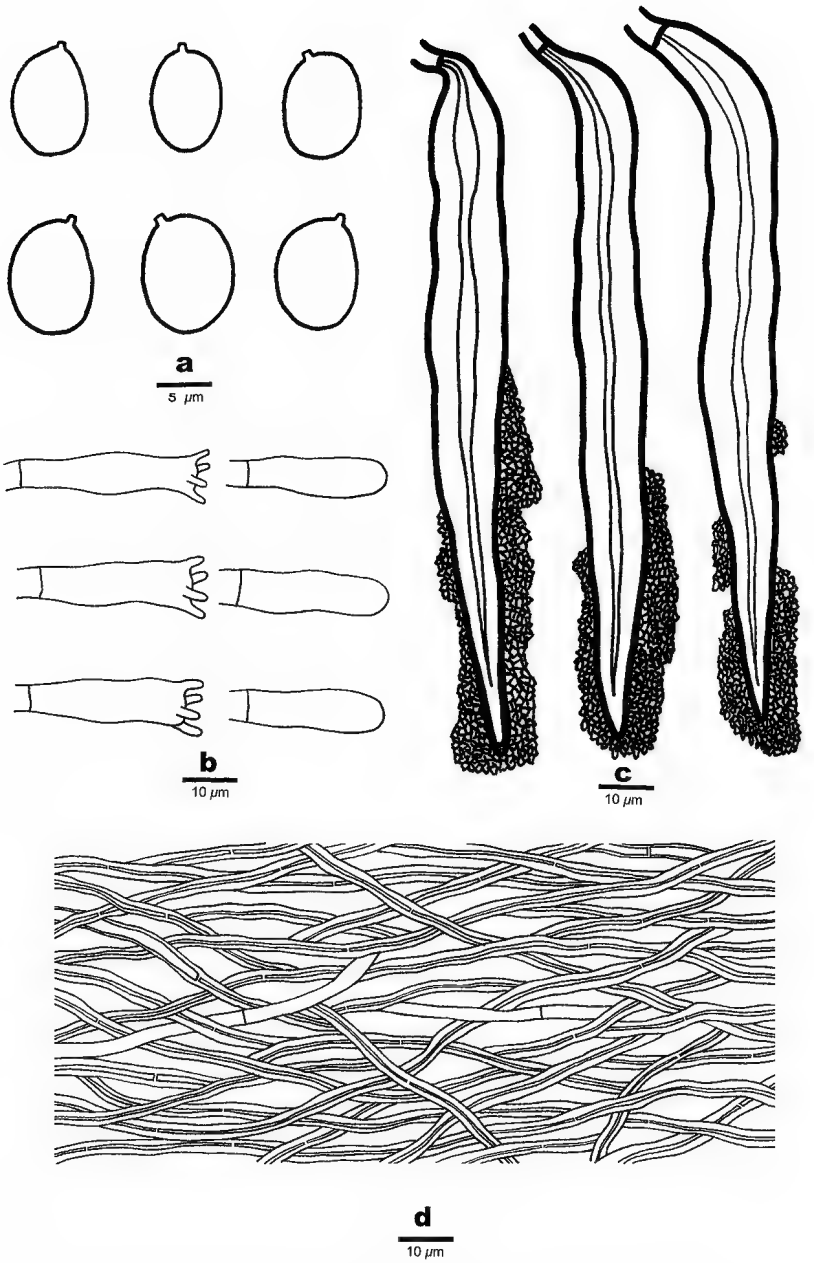

FIG. 2. Microscopic structures of *Hymenochaete megaspora* (drawn from the holotype). a: Basidiospores. b: Basidia and basidioles. c: Setae. d: Hyphae from subiculum.

μm), but *H. gigaspora* differs in having smaller setae without encrustation (75–100 × 5–10 μm), and lacking a cortex and tomentum (Léger 1998, Parmasto 2005). Another similar species, *H. tabacina* (Sowerby) Lév., which also has encrusted setae and a cortex, can be distinguished by its smaller basidiospores (4.5–7 × 1.2–2.2 μm) and presence of setal hyphae (Parmasto 2001).

Macroscopically, *Hymenochaete megaspora* is similar to *H. rigidula* Berk. & M.A. Curtis, which is another common species on *Quercus* in Xizang Autonomous Region. However, *H. rigidula* has shorter setae (40–60 × 7–12 μm) and smaller basidiospores (3.7–5 × 1.5–2.3 μm, Parmasto 2001).

Hymenochaete acerosa S.H. He & Hai J. Li, **sp. nov.** FIGS. 3–4

MYCOBANK MB 519355

Carpophorum annuum, effusum, adnatum, molle, 200–600 μm crassum. Hymenophorum laeve, azonatum, non crevisum, cinnamomeum vel flavo-brunneum. Cortex et tomentum desunt, stratum hypharum adest. Systema hypharum monomiticum, hyphae generativae non fibulatae, in subiculo hyalinae vel flavo-brunneae, crasse tunicatae, ad angulum 90° frequanter ramosae, septatae, laxe intertextae, 2–5 μm diam; setae in hymeniis stratosis numerosae, flavo-brunneae, acerosae, acutae, interdum curvatae vel leviter sigmoideae, (55–)85–170(–180) × 5–8(–9) μm; hyphidia et cystidia desunt; basidia clavata, 4-sterigmatibus praedita, 13–21 × 5–8 μm, ad basim uniseptata; sporae ellipsoideae vel late ellipsoideae, hyalinae, tenuiter tunicatae, laeves, IKI–, CB–, (6.5–)7–8.5(–9) × (4.6–)4.8–6 μm.

TYPE: China. Xizang Autonomous Region, Linzhi County, Lulang, alt 3700 m, on dead angiosperm branch, 18.IX.2010 He 344 (**Holotype,** BJFC).

ETYMOLOGY *acerosa*, refers to the acerose setae.

FIG. 3. Fruitbody of *Hymenochaete acerosa* (He 344, holotype).

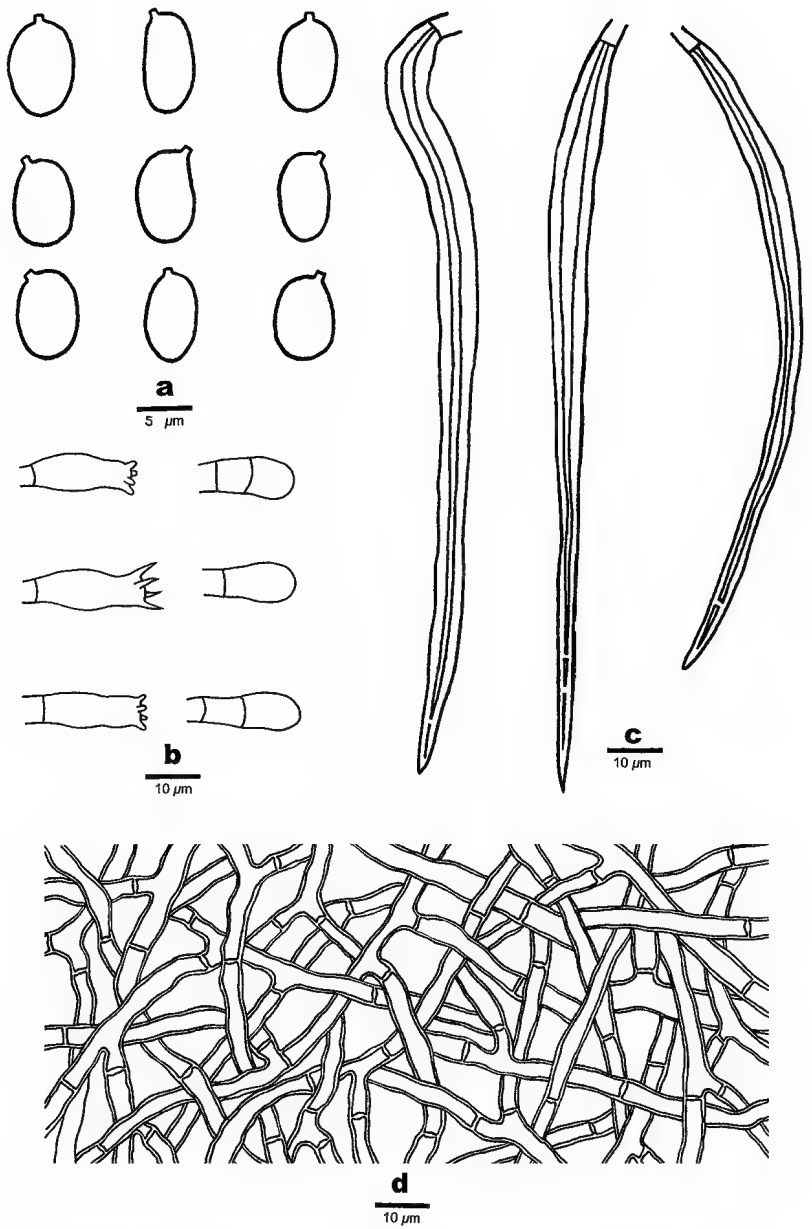

Fig. 4. Microscopic structures of *Hymenochaete acerosa* (drawn from the holotype). a: Basidiospores. b: Basidia and basidioles. c: Setae. d: Hyphae from subiculum.

FRUITBODY: Annual, effused, adnate, detachable, soft, first as small colonies, later confluent up to 15 cm or more in longest dimension, 200–600 μm thick. Hymenophore smooth, azonate, cinnamon to yellowish brown, usually not cracked; margin thinning out, indistinct, byssoid, concolorous with hymenophore.

HYPHAL STRUCTURE: Hyphal system monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

SUBICULUM: Cortex and tomentum absent, hyphal layer present. Generative hyphae hyaline to yellowish brown, thick-walled with a wide lumen, frequently branched at a right angle, with numerous simple septa, loosely interwoven, 2–5 μm in diam.

STRATIFIED HYMENIUM: Hyphae in this layer similar to those in subiculum, yellowish to yellowish brown, thick-walled, more or less agglutinated, interwoven, 2–4 μm in diam. Setae numerous, yellowish brown, acerose with acute tip, sometimes curved or slightly sigmoid, projecting up to 100 μm above the hymenium, (55–)85–170(–180) × 5–8(–9) μm; cystidia and hyphidia absent; basidia clavate, with four sterigmata and a simple septum at base, 13–21 × 5–8 μm; basidioles in shape similar to basidia, but smaller.

SPORES: Basidiospores ellipsoid or broadly ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (6.5–)7–8.5(–9) × (4.6–)4.8–6 μm, L = 7.69 μm, W = 5.30 μm, Q= 1.40–1.50 (n = 60/2).

ADDITIONAL SPECIMENS (PARATYPES) EXAMINED: **CHINA. XIZANG AUTONOMOUS REGION**, Linzhi County, Lulang, alt 3700 m, on dead angiosperm branch, 18.IX.2010 He 338; 25.IX.2010 He 399 (Paratypes, BJFC).

REMARKS: *Hymenochaete acerosa* is distinguished by the very long and narrow (acerose) setae, large basidiospores, and loosely interwove hyphae. It is similar to *H. depallens* Berk. & M.A. Curtis, which also has long setae and loosely interwove hyphae; however, the new species differs in having narrower setae (85–170 × 5–8 μm vs. 80–150 × 9–13 μm), larger basidiospores (7–8.5 × 4.8–6 μm vs. 6–7.3 × 3.7–4.2 μm), and an uncracked hymenophore (Léger 1998, Parmasto 2005).

Hymenochaete acerosa is superficially similar to *H. cinnamomea* (Pers.) Bres., which differs in having shorter setae (70–120 × 5–9 μm), smaller basidiospores (4.5–6.5 × 1.8–2.8 μm), and always stratified hyphal and setal layer (Léger 1998, Parmasto 2001).

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Rare or little known corticioid basidiomycetes from southern Belarus

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ABSTRACT — Six non-poroid resupinate homobasidiomycete species, collected within the borders of Paleskaya Physiographic Province, or Polesia (Belarus), are described and illustrated: *Crustodontia chrysocreas*, *Hyphoderma cryptocallimon*, *H. transiens*, *Oliveonia citrispora*, *Trechispora caucasica*, *T. minuta*. According to the known distributional data, these taxa are rare or exceptionally rare in Belarus and other European countries; they are, except *H. transiens*, reported from Belarus for the first time. Distinctive characters and morphological differences from similar-looking species are discussed.

KEY WORDS — *Basidiomycota*, *Corticiaceae*, geography, morphology

Introduction

This paper continues a series on rare corticioid fungi from Belarus. Two previous publications were devoted to the species found in central (Yurchenko & Kotiranta 2006) and northern (Yurchenko & Kotiranta 2007) parts of the country. The species described here were found in southern Belarus and are rare or very rare in Belarus and most of the other European countries. The morphology of these species, except *Crustodontia chrysocreas*, is relatively little documented, and thus additional descriptions are needed.

Materials & methods

The material was collected from Paleskaya Province, or province No.845 according to the European decimal physiographic division (see Klitsunova et al. 2002). In geographical literature this area is better known as Belarusian part of Polesia Lowland or Belarusian Palesse (FIG. 1). The corresponding author collected most of the specimens in 2006 and 2007; otherwise the collector's name is indicated.

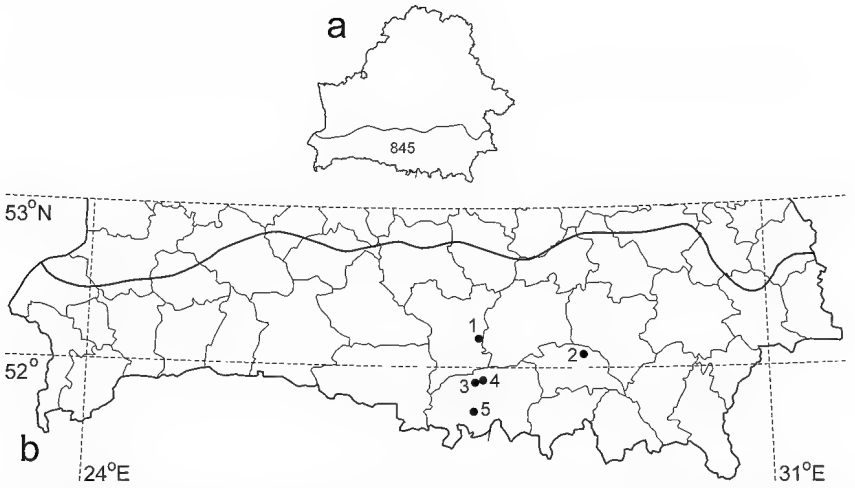

FIG. 1. Study area and collection sites: **a**, contour of the Republic of Belarus and Paleskaya Province (province No. 845, according to the European decimal physiographic division); **b**, collection sites, northern border of the Province and the borders of Belarus administrative districts (site numbers are the same as mentioned in SPECIMENS EXAMINED).

The macromorphology was described from dry samples. For micromorphological measurements and drawings the material was mounted in 3% KOH water solution or in distilled water to observe crystals; Melzer's reagent (Mz's) was used to test amyloidity or dextrinoidity, and Cotton Blue (CB; 0.1% Cotton Blue in 60% lactic acid) to test for cyanophily. The studied samples are deposited in the Herbarium of V.F. Kuprevich Institute of Experimental Botany (MSK-F); duplicates are also deposited in the reference herbarium of Heikki Kotiranta (H.K.) and one in the University of Oslo Herbarium (O).

Species descriptions

Crustodontia chrysocreas (Berk. & M.A. Curtis) Hjortstam & Ryvarden FIG. 2

BASIDIOMA totally effused, closely adnate, about 10 × 5 cm, 50–100 µm thick in dry state, ceraceous or subcartilaginous, somewhat fissured in dry state; hymenophore greyish ochraceous with orange tint, under the lens slightly pruinose, with the naked eye like grayish bloom, smooth or with minute, quite scattered, blunt warts. MARGIN concolorous, thinning out. SUBICULUM rather thin and subhymenium thick; subhymenium with pigmented resinous inclusions. HYPHAL SYSTEM monomitic, hyphae clamped at all septa. Subicular hyphae moderately branched, more or less sinuous, even or with swellings, 2.2–4.1 µm wide, hyaline, thin- to slightly thick-walled, smooth. Subhymenial hyphae densely branched, short-celled, densely packed and forming a pseudoparenchymatic tissue, 2–3 µm wide, with swellings up to 4 µm wide,

hyaline, thin- or slightly thick-walled, smooth. HYMENIUM consisting of densely palisade-arranged cells, with brownish resinous excretions in between. CYSTIDIOLES (leptocystidia) fusoid, blunt, about 20 µm long, 2.3–4.3 µm wide, projecting 6–8 µm above the hymenium, hyaline, thin-walled, naked or apically with capitate, pale brownish or yellow encrustations, 2.2–6.7 µm in diam. BASIDIA cylindrical or narrowly clavate, 16–21 × 3.8–4.2 µm, hyaline, thin-walled, with 4 sterigmata 6–6.5 × 0.8 µm. BASIDIOSPORES narrowly ellipsoid, (3.7–)4.2–4.7(–5.5) × (2–)2.2–2.3 µm, mostly slightly thick-walled, hyaline or subhyaline, with very small apiculus, Mz's-negative, acyanophilous.

SPECIMEN EXAMINED **BELARUS. Homel' voblast':** Lel'chytsy district, in vicinity of Baravoe village (site 5), on decorticated fallen wood of *Quercus robur* L. in *Quercus* forest of *Vaccinium myrtillus* type, coll. E.P. Komarova 22 VIII 1963 (MSK 10574; H.K.; O).

Crustodontia chrysocreas is distinguished by its slightly warted hymenial surface, usually ochraceous hymenium with orange and brownish hues, short-celled, and tightly packed hyphae in textura intricata, which gives the basidioma a cartilaginous consistency (Lombard et al. 1975 as '*Phlebia chrysocrea* (Berk. & M.A. Curtis) Burds.', Domański 1991 as '*Ph. chrysocreas*'). Our specimen fits *C. chrysocreas* in colour, hymenial configuration, basidioma texture, leptocystidia, basidia, and spore morphology. However, we did not observe microbinding hyphae or the waxy, yellow substance described by Lombard et al. (1975) in the substratum under the basidioma. Moreover, no parts of the fungus turned red in 3% KOH, which is characteristic for American (Lombard et al. 1975), Hawaiian (Gilbertson & Adaskaveg 1993), and Japanese (Maekawa 1993) material. Microbinding hyphae were not observed in Asian material (Wu 1990 as '*Amethicium chrysocreas* (Berk. & M.A. Curtis) Sheng H. Wu', Maekawa 1993). Abundant yellowish (but not orange brown, as in the American samples) granular material was found in subhymenium and especially hymenium, but absent in subiculum. The colour differences and the absence of KOH reaction is probably due to the long-time storage of the specimen in herbarium. Remarkably, in his original description of *Corticium chrysocreas* Berk. & M.A. Curtis, Berkeley stated, "subiculum bright yellow" (Berkeley 1873: 178), but Maekawa (1993) described the fungus as pale yellow in section. Our sample has smaller (although similarly shaped) cystidia compared with those described by Lombard et al. (1975) and Maekawa (1993). The material described from Hawaii (Gilbertson & Adaskaveg 1993) had oblong to cylindrical spores and remarkable hyphal elements with vesicular apices up to 22 µm wide, which are absent in our sample.

The species is new for Belarus and the record is the northernmost (51.7°N) known in the world. It has a pantropical distribution and is exceptionally rare in Europe, earlier collected in France (Bourdot & Galzin 1928), Poland, and

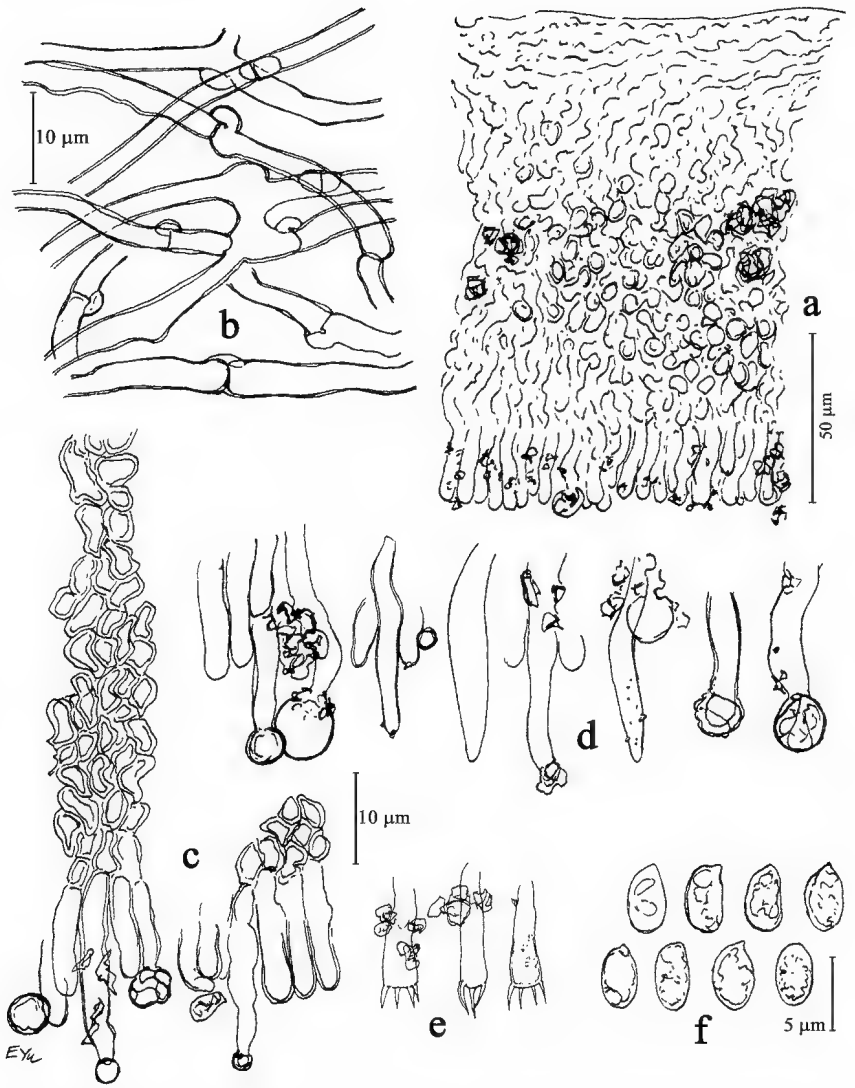

FIG. 2. *Crustodontia chrysocreas* (MSK 10574): **a**, vertical section through basidioma; **b**, subicular hyphae close to the substratum; **c**, portions of hymenium and subhymenium; **d**, cystidia and basidioles; **e**, basidia; **f**, basidiospores.

Ukraine (Bernicchia & Gorjón 2010). The species has been reported from southeastern and eastern USA (Lombard et al. 1975 – 10 localities), Costa Rica (K.-H. Larsson 2010, pers. comm.), Caribbean Islands (Minter et al. 2002 – 18 records), Venezuela (Minter 2010), six countries of equatorial Africa

(Hjortstam et al. 1993, Roberts 2000), Sri Lanka (Lombard et al. 1975), southern and southeast China (Dai et al. 2004, Dai 2010), Taiwan (Lin & Chen 1990, Wu 1990), Japan (Maekawa 1993 – 8 localities; Kubayashi & Maekawa 2001), Hawaii (Gilbertson & Adaskaveg 1993), Brunei (Hjortstam et al. 1998), western Australia (Hjortstam et al. 2009), New Zealand (Pennycook & Galloway 2004). We suppose, in accordance with Hjortstam et al. (2009), that the material from different isolated landmasses is heterogeneous, comprising several species, which is reflected in differences in descriptions.

Hyphoderma cryptocallimon B. de Vries FIG. 3

BASIDIOMA effused, closely adnate, subceraceous, several cm long, 0.1–0.2 mm thick, smooth and continuous to porulose, especially towards the margin, cream- or pale coloured. MARGIN diffuse. HYPHAL SYSTEM monomitic. Subicular hyphae clamped, moderately branched, 2–3.7 µm wide, hyaline, thin-walled. CYSTIDIA scattered, projecting up to 65–100 µm above the hymenium, cylindrical or with some dilatations towards the base and/or to the apex, apically occasionally subcapitate or with a small resinous cap, non-septate or seldom with 1–3 adventitious septa, 70–90(–125) × 5.5–9.2 µm, hyaline, apically thin-walled, at the base slightly or distinctly thick-walled (to 1 µm). HYPHIDIA numerous in some hymenial areas, cylindrical, hyaline, thin-walled, straight to sinuous, 1.5–2.3 µm wide, projecting up to 5–15 µm. BASIDIOLES often rich in oil-drops. BASIDIA subcylindrical or narrowly clavate, hyaline, thin-walled, rich of oily inclusions, (30–)45–50 × 7–10.3 µm, with (2)4 sterigmata 6.5–9 × 1.3–1.7 µm, some sterigmata basally with an oily inclusion, with attenuated to hair-like tip when mature. BASIDIOSPORES narrowly ellipsoid to oblong, sometimes narrowing to the base, (8.5–)9.5–11(–13.5) × (4.5–)5–5.5(–6.7) µm, hyaline, often with a large, strongly refractive oil drop, thin-walled, smooth, with rather narrow, distinct apiculus, inamyloid, acyanophilous.

SPECIMEN EXAMINED **BELARUS. Homel' voblast'**: Lel'chytsy district, in vicinity of Simanichy village (site 3), on fallen decorticated branch of *Pinus sylvestris* L. in *Pinus* forest of *Pleurozium* type, coll. 9.XI.2007 (MSK 6904; H.K.). The fungus is mixed with *Tubulicrinis subulatus* (Bourdot & Galzin) Donk basidioma, and grew over it.

Eriksson & Ryvarden (1975: 543) first diagnosed this taxon under the name *Hyphoderma* sp. de Vries 488, based on a single sample from northwest Germany. Nakasone & Gilbertson (1982: 603–605) described a new species in the genus *Crustoderma*, *C. opuntiae* Nakasone & Gilb., from Arizona, with the notation that it is morphologically similar to *Hyphoderma* sp. de Vries 488. However, we see several differences between the *C. opuntiae* and *H. cryptocallimon* diagnoses that do not support their synonymy: *C. opuntiae* has floccose subiculum with moderately thick-walled hyphae, somewhat flexuose cystidia, narrower basidia (6–7 µm) of narrowly clavate shape, narrower spores (4–5 um wide), and

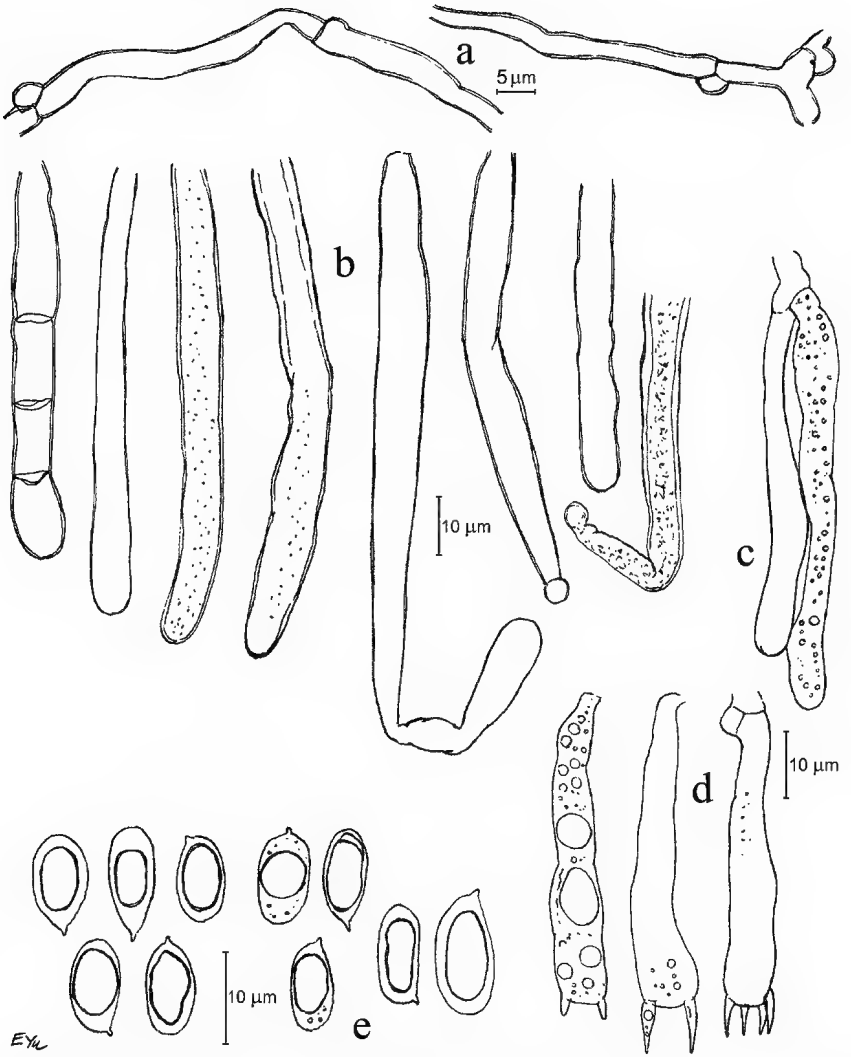

FIG. 3. *Hyphoderma cryptocallimon* (MSK 6904): **a**, subicular hyphae; **b**, cystidia; **c**, basidioles; **d**, basidia; **e**, basidiospores.

negative oxidase test, indicating a brown rot activity, whereas the members of the genus *Hyphoderma* are white rot fungi.

Hyphoderma cryptocallimon is morphologically close to *H. obtusiforme* J. Erikss. & Å. Strid, differing from it by shorter basidiospores, much longer and projecting cystidia that are often distally slightly clavate and basally with thickened or thick walls and sometimes with adventitious septa, and somewhat

larger and slightly more sinuous basidia (25–70 × 7–10 µm), usually with many guttules (Eriksson & Ryvarden 1975, de Vries 1987). Cystidia in *H. obtusiforme* are 50–80 µm long, thin-walled and not subclavate; besides, they often have dense protoplasm in the distal part. The spore size in *H. cryptocallimon* is noted as 7–12 × 4.5–7 um, whereas spores are 10–14 × 5–7 µm in *H. obtusiforme* (Eriksson & Ryvarden 1975, de Vries 1987). There is also a presumed ecological distinction: *H. cryptocallimon* prefers habitats on poorer sandy soils, which is seen at least from the finds described by de Vries and from our finding. Some spores in our material were longer than 12 µm, thus approaching the spore size of *H. obtusiforme*. Moreover, our sample has many cylindrical cystidia, only some of which expanded slightly toward the apex; some cystidia are subcapitate, uncommon for *H. cryptocallimon*.

The species is new for Belarus and known from a single locality. *H. cryptocallimon* was described on *Juniperus communis* from northwest Germany and reported also from southern Sweden and the Netherlands (de Vries 1987), Italy on *Juniperus* and *Pinus* (Bernicchia 2000, Bernicchia et al. 2007a), Zakarpats'ka oblast in Ukraine (Küffer et al. 2004), Britain including Northern Ireland (Kirk & Cooper 2009), and Belgium and Spain (Bernicchia & Gorjón 2010).

Hyphoderma transiens (Bres.) Parmasto FIG. 4

BASIDIOMATA totally effused, more or less closely adnate, 5 cm or more in extent, 50–130 µm thick between the teeth, subceraceous, cream-coloured. HYMENOPHORE odontioid, at basidioma periphery partly smooth or minutely porulose; teeth tuberculate-flattened at the periphery of the basidioma, in the center conical or cylindrical, brownish or dark ochraceous, glossy, fragile, up to 0.4 mm long and 0.2 mm wide, simple or slightly branched, obtuse or acute, smooth or apically short-setaceous; with age or when dry basidioma minutely cracked. MARGIN farinaceous, diffuse, in some areas almost mould-like (short-fibrillose), whitish or cream-coloured, 0.3–0.7 mm wide. HYPHAL SYSTEM monomitic, hyphae clamped at all septa. Subicular hyphae moderately branched, more or less loosely interwoven, 2–3.7 µm wide, hyaline, thin- to slightly thick-walled, smooth or sparsely encrusted (in KOH). Tooth trama with abundant yellowish, coarse crystalline material (in water); apical hyphae of the teeth often forming clusters, individual hyphae 1.3–3.5 µm wide. Subhymenial hyphae moderately branched, more or less vertically arranged, 2–2.5 µm wide, hyaline, thin-walled, richly covered with aggregations of yellowish coarse crystalline material, not dissolved in KOH. CYSTIDIA rare or scattered, more or less embedded, subcylindrical, 40–95 × 7–9(–10) µm, subhyaline, thin-walled, in KOH smooth. BASIDIA irregularly clavate, 25–30 (–38) × 5.7–6.7 µm, hyaline, thin-walled, smooth or basally encrusted, with 2–4 sterigmata, initially stout and obtuse, when mature conical or subulate, 6.5–9.5

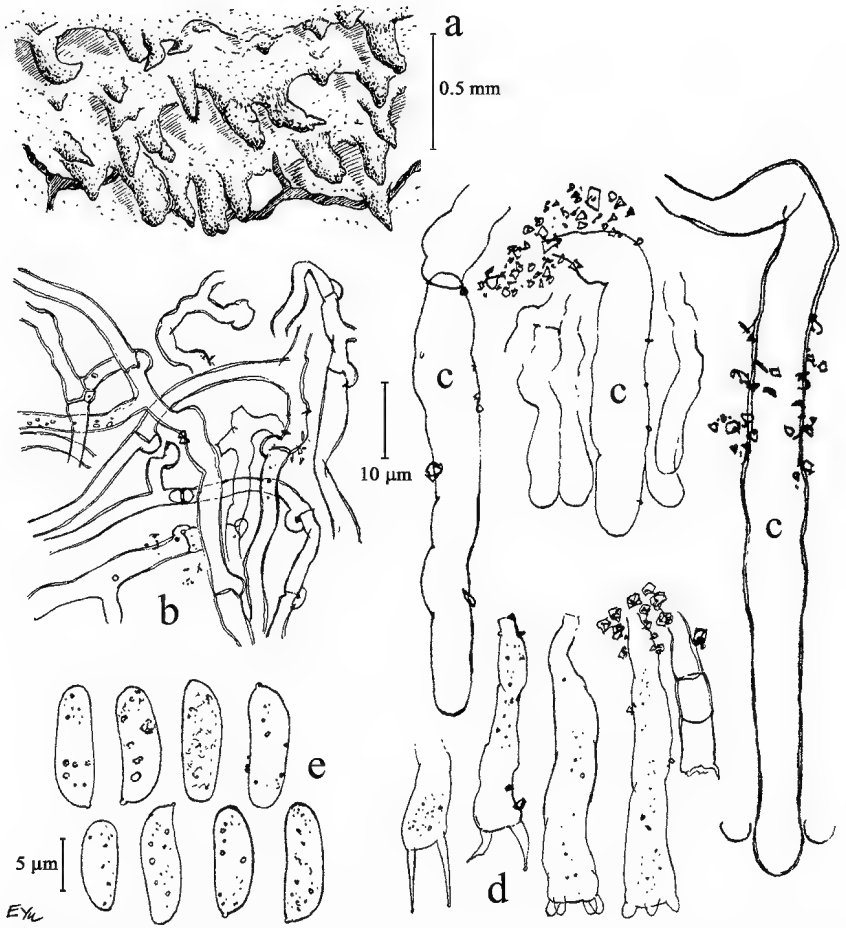

FIG. 4. *Hyphoderma transiens* (MSK 3965): **a**, view of odontioid hymenophore; **b**, subicular hyphae; **c**, cystidia; **d**, basidia; **e**, basidiospores.

× 1.5–1.8 µm. BASIDIOSPORES cylindrical, adaxially straight or slightly concave, occasionally gently sigmoid, 8.5–12 × 2.7–4.1 µm, thin-walled, colourless, with small dispersed oil drops, with short and blunt, sometimes not pronounced apiculus, Mz's-negative, acyanophilous.

SPECIMENS EXAMINED **BELARUS. Homel' voblast'**: Zhytkavichy district, in the vicinity of Naida village (site 1), on fallen corticated branch of *Corylus avellana* L. and unidentified deciduous tree in *Alnus glutinosa* forest of *Urtica* type, coll. A.I. Golovko 21.VIII.1967 (MSK 10505a, 10505b); Lel'chytsy district, in the vicinity of Baravoe village (site 5), on fallen decorticated wood of *Populus tremula* L. in *Alnus glutinosa* forest of *Sphagnum* type, coll. E.P. Komarova 17.VIII.1963 (MSK 3965).

Distinctive characters of *H. transiens* are the odontioid hymenophore, which smoothens towards the basidioma periphery, crystal-rich aculeal trama, the presence of scattered, almost immersed subcylindrical cystidia (Maekawa 1994), and relatively narrow basidiospores. The hymenophore colour is usually pale ochraceous and varies little even between samples from different continents (Hjortstam & Ramos Bononi 1987, Maekawa 1994). Other authors (Nikolajeva 1961 as '*Odontia transiens* Bres.', Hjortstam & Ramos Bononi 1987, Maekawa 1993: 12 as '*H. longosporum* (H. Furuk.) N. Maek.', 1994: 65) noted longer basidiospores (≤ 13–15 µm). Nikolajeva (1961) described also cylindrical, spindle-shaped or slightly capitate apically encrusted (rough) cystidia, which were not observed by us. The same author described the hymenophore as warted with warts having penicillate apices, whereas our material is irregularly warted to irregularly short-aculeate, with barely pronounced or absent penicillate projections at aculei apices. A morphologically close species is *H. roseocremeum* (Bres.) Donk, but it differs by a smooth hymenophore.

Hyphoderma transiens was published for the first time from Belarus from the northwestern part of the country (Yurchenko 2008). Two other known sites are in the southern part, but the species has not been recollected there since 1967. The species is common in the Mediterranean and frequent on *Quercus* in Italy (Bernicchia et al. 2007b, 2008) and has been reported from many other warm regions: southern Brazil (Hjortstam & Ramos Bononi 1987), Azores (Telleria et al. 2009a,b – common), Madeira (Telleria et al. 2008), Krasnodar krai of Russia, Georgia, Azerbaijan, northeast Turkey, northern Iran (Ghobad-Nejhad et al. 2009), north-central China (Dai et al. 2004, Dai 2010), Japan (Maekawa 1994). In the more northern areas there are records from the Carpathians and the Forest Steppe Zone of Ukraine (Nikolajeva 1961, Zerova et al. 1972 – under the name '*Odontia transiensis*'), and Middle Volga Region in Russia (Malysheva & Malysheva 2008). Bernicchia & Gorjón (2010) cite the northernmost localities as Sweden and Estonia.

Oliveonia citrispora (Hauerslev) P. Roberts FIG. 5

BASIDIOMA totally effused, 1–7 mm long, ca 30 µm thick, hypochnoid, comparatively loosely attached to the substratum, not continuous, porulose-reticulate, greyish with yellowish or brownish tint. MARGIN rather abrupt or indistinct. SUBICULUM poorly developed; subicular hyphae moderately branched, with small clamps or rarely with simple septa, (1.3–)1.6–2(–2.8) µm wide (some segments inflated to 4 µm), thin-walled, smooth, hyaline or with pale yellowish refractive contents. Subbasidial hyphae moderately branched, 1.8–2(–3) µm wide, thin-walled, hyaline or with yellowish contents, with clamped septa. CYSTIDIA none, but broadly fusoid cystidioles scattered, 12–17.5 × 3.2–4.7 µm, yellowish. BASIDIOLES broadly clavate, narrowly ovoid

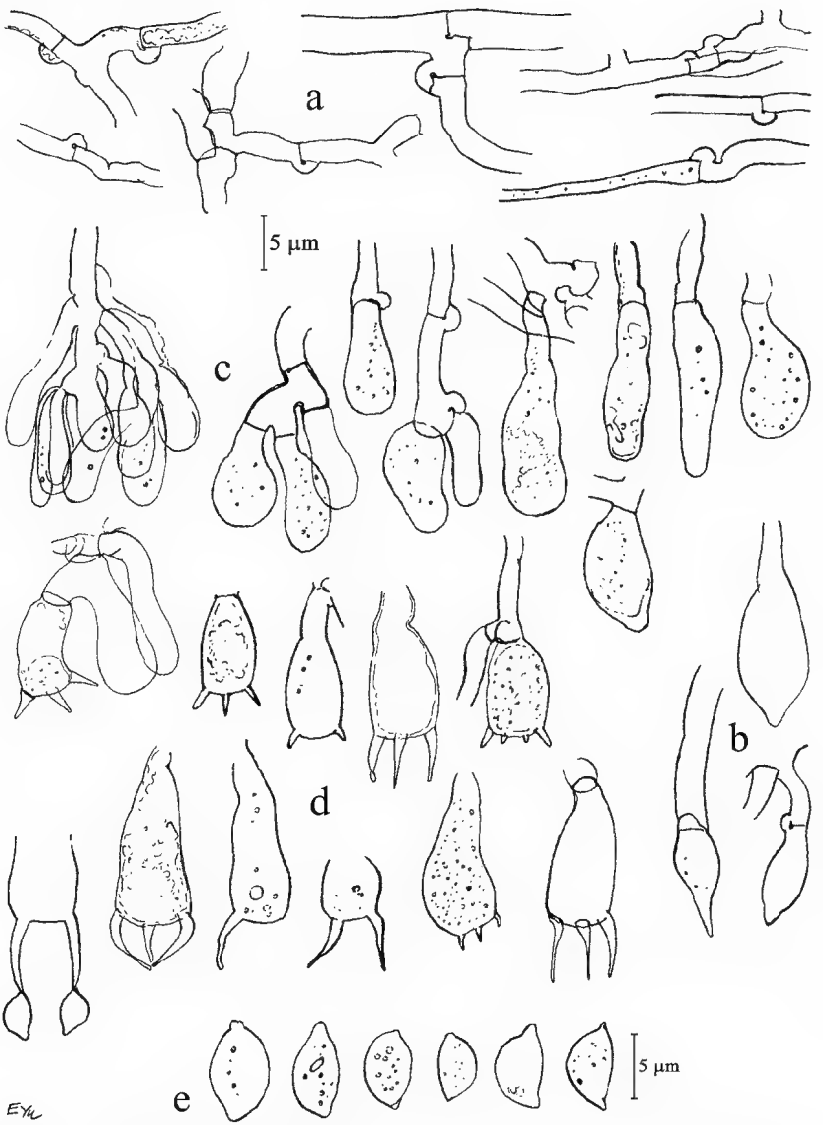

FIG. 5. *Oliveonia citrispora* (MSK 6895): **a**, subicular hyphae; **b**, broadly fusoid cystidioles; **c**, basidioles and basidia; **d**, basidiospores.

or subcylindrical, 11–25 × 5.7–7.5(–8.5) µm, with subhyaline to brownish yellow, minutely granular or refractive contents. BASIDIA ovoid to broadly clavate, basally clamped (but the clamp not always seen), 10–16.5 × 4.5–7.7 µm, hyaline or pale yellowish, thin-walled (wall thinner basally), with 1–4

sterigmata, 2.2–6.5 × 0.8–1.1 μm. Basidiospores wide-navicular – citriform, narrowing towards both ends, (4.3–)6–8.2 × 2.9–4.3 μm, thin-walled, smooth, hyaline to yellowish, with short blunt apiculus, Mz's-negative, acyanophilous.

Specimens examined **BELARUS. Homel' voblast'**: Lel'chytsy district, Prypyatski National Park, in the vicinity of Simanitskaya Rudnya village (site 4), on decorticated wood of strongly decayed *Picea abies* (L.) H. Karst. stump in *Betula-Picea* forest of *Vaccinium myrtillus* type, coll. 9.XI.2007 (MSK 6895; H.K.); the same locality, on decaying *Picea abies* bark in *Picea* forest of swampy moss type, coll. 21.V.2010 (MSK 7291).

Oliveonia citrispora is distinguished from other members of the genus by bi-apiculate basidiospores. Our material deviates somewhat compared with other descriptions of the species. According to Roberts (1999), *O. citrispora* has narrower (1.5–2 μm) hyphae, subglobose, cuboid or ellipsoid basidia that are shorter (7.5–11 μm) and often pleural, larger sterigmata (4–10 × 1–1.5 μm) and larger basidiospores (6–10 × 3.5–8 μm) with pronounced apiculi. However, such small differences are common in *Rhizoctonia*-forming fungi. Basidiospores in our material are notably narrow, even when compared with the data by Roberts (1999: Tab. 30) for a sample with the narrowest spores (up to 6 um). Our samples have a dirty yellowish pigmentation in hymenial elements and hyphae, giving a yellowish or brownish tinge to the hymenial surface, whereas basidiomata are greyish-white according to other descriptions (Hauerslev & Roberts 1997 as '*Sebacinella citrispora* Hauerslev', Roberts 1999). Spore repetition is often found in this taxon (Hauerslev & Roberts 1997) but was not detected in the specimens examined here.

The species is new for Belarus and known from a single locality. It is probably rare over its entire small range: so far it is known from the Netherlands, Denmark (Roberts 1999), southern Britain (Kirk & Cooper 2009 – 7 records), and Sweden (Bernicchia & Gorjón 2010).

Trechispora caucasica (Parmasto) Liberta Fig. 6.

Basidiomata totally effused, ca 5–15 mm long, very thin, closely adnate but not firmly attached to the substratum, pure white, arachnoid-reticulate, with scattered loose granules, 0.2–1 mm in diam., containing conidia or hyphae with abundant coarse crystalline material. Margin abrupt or indistinct. Hyphal strands present, 70–150 μm wide, situated near the basidiomata or radiating in flabelliform pattern from the margins. Hyphal system monomitic, hyphae clamped. Subicular hyphae moderately branched, 1.3–1.8 μm wide, hyaline, thin-walled, in strands straight, 1–1.5 μm wide. Subhymenium rich of fine crystalline material and also with groups of coarse crystals (separate crystals 2.5–11 μm across). Subbasidial hyphae moderately branched, 1.6–2(–3.5) μm wide, hyaline, thin-walled, moderately encrusted or smooth. Cystidia none. Basidia basally clamped, short clavate to subcylindrical, some with a median

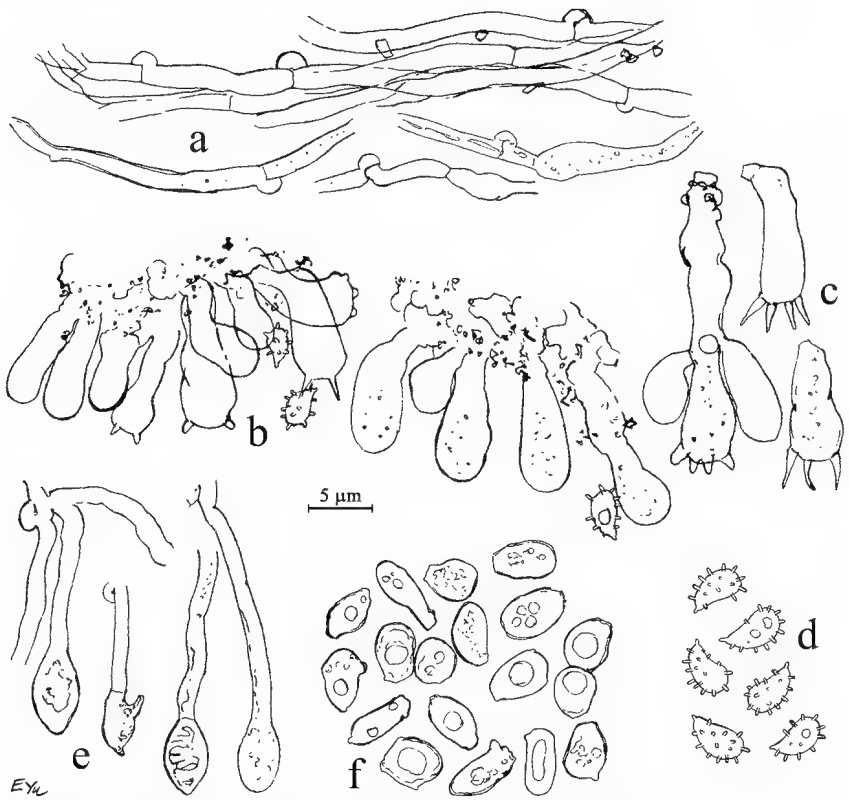

FIG. 6. *Trechispora caucasica* (MSK 6891): **a**, subicular hyphae; **b**, fragments of hymenium; **c**, basidia and basidioles; **d**, basidiospores; **e**, conidiophores with developing conidia; **f**, conidia.

constriction, thin-walled, hyaline, 9–11.5(–18) × 4–4.5(–5) µm, with 2–4 sterigmata, 2.5–3.2 × 0.5 µm. BASIDIOSPORES ovoid, somewhat bent, adaxially concave, 3.3–4(–5.5) × 2.5–2.7 µm, relatively evenly covered with spines up to 0.8 µm long, thin-walled, hyaline, with middle-sized apiculus, Mz's-negative, acyanophylous. CONIDIA (blastoconidia) numerous, originating terminally, sometimes intercalarly, on delicate hyphae, 1–2 µm wide, ellipsoid, ovoid, angular-subglobose or irregular-shaped, 4.2–6.2 × 2.2–4 µm, smooth, or with one, or a few protuberances, with thickened walls, subhyaline, with one large drop or several smaller inclusions.

SPECIMEN EXAMINED – **BELARUS. Homel' voblast'**: Lel'chytsy district, Prypyatski National Park, in the vicinity of Simanichy village (site 3), on corticated fallen branch of *Pinus sylvestris* in *Pinus* forest of *Pleurozium* type, coll. 9.XI.2007 (MSK 6891; H.K.).

The presence of hyphal cords, lack of skeletal hyphae, often irregular shape of conidia, and especially morphology of basidiospores, which are ventrally

somewhat concave and bearing comparatively long aculei, are characteristic for *T. caucasica* (Parmasto 1965, Larsson 1992). A very similar species is *T. tenuicula* (Litsch.) K.H. Larss., which, however, is dimitic, grows mostly on ferns (whereas *T. caucasica* grows on wooden substrata), and spores are ellipsoid to nearly lacrymiform, densely aculeate, with a small spine-free area on the ventral side (e.g., Larsson 1992, Kotiranta & Saarenoksa 2000).

Trechispora caucasica is new for Belarus and known from a single locality. The species was described for the first time from Azerbaijan (Parmasto 1965, as '*Cristella caucasica* Parmasto'), but today it is known from Britain (Kirk & Cooper 2009 – 2 records), Norway (Ryvarden et al. 2003 – from 3 localities), Finland (Kotiranta et al. 2009 – 2 localities), Lithuania (R. Iršėnaitė 2010), Sweden, Denmark, France, Austria, Italy, USA (Larsson 1992), Azores (Telleria et al. 2009b). It is a rare or probably under-reported taxon, as earlier it was not distinguished from *T. farinacea* (Pers.) Liberta s. l. (Liberta 1973, Hjortstam 1987, Domański 1992). However, Liberta (1973) did not describe blastoconidia or aleuriospores, typical of *T. caucasica*, in *T. farinacea* s.l. The distinction of *T. caucasica* from *T. farinacea* was made clear after the treatments by Larsson (1992; 1996).

Trechispora minuta K.H. Larss. FIG. 7

BASIDIOMATA totally effused, closely adnate, very thin (about 15–20 µm thick), discontinuous, farinaceous or pruinose, 3–10 mm in extent, white. MARGIN diffuse, sometimes with very slender, closely adpressed, fan-shaped hyphal strands at the periphery. HYPHAL SYSTEM dimitic. Generative hyphae clamped, moderately branched, hyaline, thin-walled, smooth to slightly encrusted, (0.9–)1.3–2.3 µm wide, subbasidial ones 1.6–1.8(–4.2) µm wide. Hyphae in cords with blunt, cystidia-like hyphal ends up to 3.7–4.3 µm wide. Skeletals not present in all parts of basidiomata, but in some areas abundant, very sparingly branched, rather straight to wavy, hyaline, 0.5–1.1(–1.8) µm wide, with lumen about 0.3 µm wide or seemingly without lumen, gradually turning into narrow generative hyphae with clamps. Skeletals often covered with numerous, coarse (mostly 3–10 µm across) crystals. HYPHIDIA occasional, sinuous, 18–32 µm long, often swollen at base to about 4 µm. BASIDIA basally clamped (clamps often obscured by fine crystalline material), shortly clavate to oblong and slightly pedunculate, straight or curved, 7.5–9.2 × (3.3–)3.5–4 µm, with 4 subulate sterigmata 3.3(–4) µm × 0.5 µm. BASIDIOSPORES ellipsoid or ovoid, with flat or convex ventral side, minutely echinulate to short aculeate, 3.3–3.8(–4.5) × 1.7–2.1 µm excluding the aculei, hyaline, thin- or rather thin-walled, Mz's-negative, acyanophilous, with minute or indistinct apiculus.

SPECIMEN EXAMINED **BELARUS. Homel' voblast'**: Mazyr district, NW outskirts of town Mazyr (site 2), in ravine bottom, on angiosperm twig in litter in *Alnus glutinosa* forest of *Rubus idaeus-Oxalis* type, coll. 26.X.2006 (MSK 7307; H.K.).

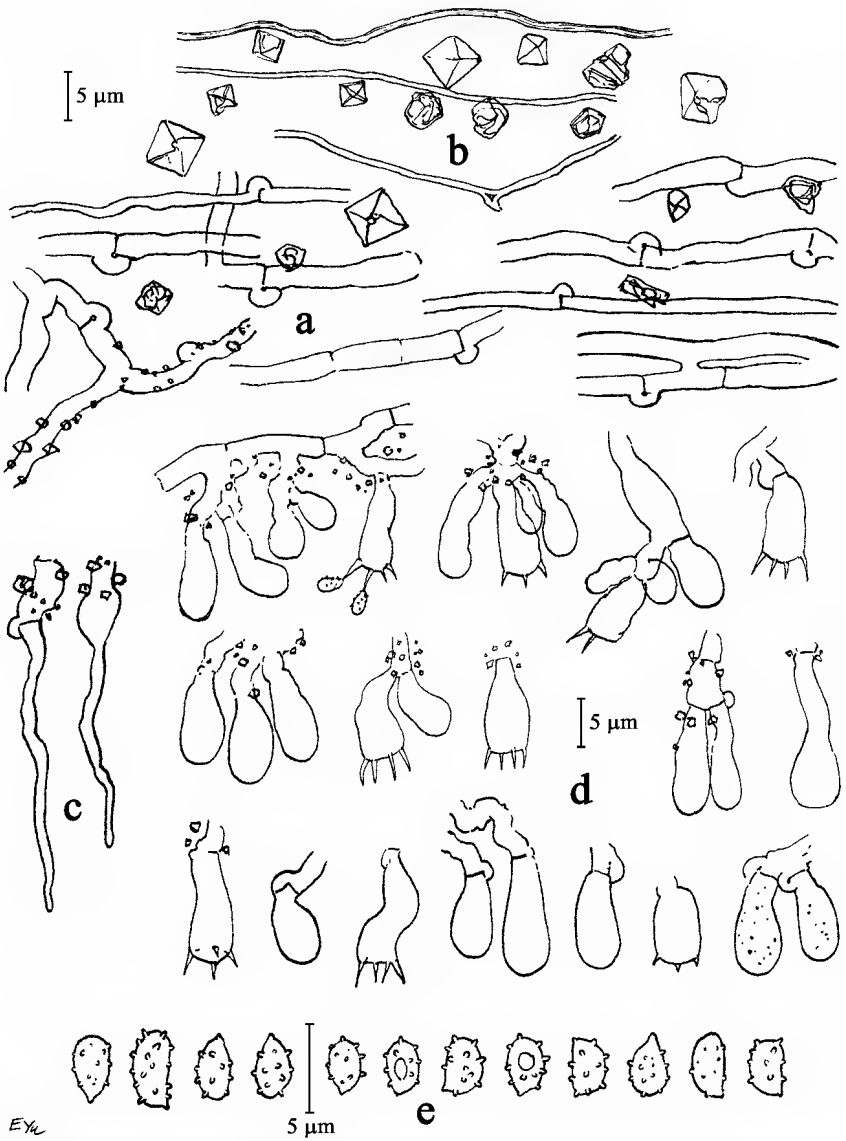

FIG. 7. *Trechispora minuta* (MSK 7307): **a**, generative hyphae; **b**, skeletal hyphae and crystals; **c**, hyphidia; **d**, basidioles and basidia; **e**, basidiospores.

The main distinctive characters for *T. minuta* are the presence of skeletal hyphae, usually pruinose to farinaceous basidiomata, very short basidia (≤ 10 × 5 µm), and a subhymenium of intricately branched hyphae in fully mature specimens

(Larsson 1992). *Trechispora tenuicula* differs in having longer basidia (10–13 μm) and the presence of blastoconidia. In contrast to the original description of *T. minuta*, our sample has an undeveloped subhymenium and narrower basidiospores with rather evenly distributed aculei. According to Larsson (1992, Fig. 28) spores are 2.4–2.7 μm wide without aculei and have almost a smooth area on the ventral side near the apiculus.

The species is new for Belarus and known from a single locality. It is widely distributed and presumably common, but is evidently neglected or often misidentified. The species is known from Norway (10 localities), Sweden (7 localities), Finland, France, Austria, Poland (at the eastern border close to Belarus), Canada, USA (Larsson 1992), and Azores (Telleria et al. 2009b).

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Conidial morphology changes in four *Phyllosticta* species

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Abstract — Production of pycnidia and conidia by *Phyllosticta theacearum*, *Phyllosticta* sp. (ex *Euonymus*) and *Phyllosticta* sp. (ex *Portulaca*) (grown on PDA), and *P. cruenta* (from specimens) was studied. On PDA, conidial appendages of *Phyllosticta* sp. (ex *Portulaca*) disappeared completely after 16 days, of *P. theacearum* after 29 days, and of *Phyllosticta* sp. (ex *Euonymus*) after 14 days. In slide preparations mounted in water, appendages of all four species persisted; the appendages of *P. cruenta* and *Phyllosticta* sp. (ex *Euonymus*) became 2 4 times longer within 30 minutes, while those of *Phyllosticta* sp. (ex *Portulaca*) and *P. theacearum* remained unchanged.

Key words — caducous, extend, identification, anamorph, *Ascomycota*, *Phoma*

Introduction

Species of the anamorphic ascomycete genus *Phyllosticta* Pers. have often been recorded as plant pathogens, saprobes, and endobionts (Liu & Lu 2007, Okane et al. 2003, Punithalingam 1974). Identification is highly problematic, as few characters are available to separate different species. Sometimes a single difference in conidial morphology, such as thickness of the mucoid layer surrounding the conidium or appendage shape or size, has been used to distinguish different species (Motohashi et al. 2008a,b, Nag Raj 1993, Punithalingam & Woodhams 1982, Aa & Vanev 2002, Wulandari et al. 2009). There have also been frequent confusions between *Phyllosticta* and *Phoma*, which have many morphological similarities. The key distinctions between these two genera are the mucoid sheath and appendage, reported for *Phyllosticta* but not for *Phoma*. Those two structures are, however, delicate, faint, colourless, and easily overlooked under the poorly contrasting conditions of bright field microscopy. In the past, that has made misidentifications between *Phyllosticta* and *Phoma* frequent. With improvements in the quality of microscopes and the wide availability of phase contrast and differential interference contrast optics, it is now easier to detect mucoid sheaths and appendages. But there is

an additional problem: in some species of *Phyllosticta* the appendages can be caducous even in fresh material and cultures (Aa 1973).

The objectives of the present paper are therefore to report preliminary observations about the time span during which appendages can be observed, and to record some changes that can occur in appearance of appendages during that period. As the species used in this study are not well documented, the opportunity is also taken to provide illustrations and descriptions of appearances in pure culture.

Materials & methods

Isolates were obtained using standard phytopathological techniques, and for three species pure cultures were established (*P. cruenta* failed because of contamination by bacteria) and incubated at 28°C in the dark. Slants on PDA were preserved at 4°C in a refrigerator. Fungal identifications were based on specific literature such as the contributions as Aa (1973), Aa & Vanev (2002) and Nag Raj (1993) among others. All slides were prepared using sterile technique. Observations of pycnidia and conidia were made on subcultures derived from original slants. Starting from 5 days after making the subculture, each Petri dish was examined daily for pycnidia. When pycnidia were detected, a slide was prepared to check for presence of conidia. The date of first appearance of conidia was then noted. From first appearance of conidia, slides were prepared daily to observe appendages. Two key dates were noted: that on which at least some conidia no longer had appendages, and that on which no conidia remained with visible appendages. Thereafter observations were discontinued. Squash mounts of pycnidia were also prepared in water to observe conidial appendages. Each slide prepared in this way was then examined at three minutes intervals for 30 minutes, and the condition of appendages noted and photographed using bright field, phase contrast, and differential interference contrast microscopy. Where no change in appendage appearance was noted, the slide was then placed on moist filter paper in a Petri dish and re-observed every 30 minutes up to 3 hours. Material that still showed no change was then re-observed daily for three days.

Specimens examined *Phyllosticta cruenta* (Fr.) J. Kickx f.: **CHINA, Shandong:** Kunyu Mountain, in diseased leaves of *Polygonatum odoratum* var. *pluriflorum* (Miq.) Ohwi (*Convallariaceae*), 37°17'04.76"N, 121°44'49.24"E, 284 m, 12 July 2007, MHQAU0134.

Phyllosticta theacearum Aa: **CHINA, Shandong:** Laoshan Mountain, in diseased leaves of *Thea sinensis* L. (*Theaceae*), 36°11'00.42"N, 120°40'49.50"E, 79 m, 25 April 2008, MHQAU0192, JJ196.

Phyllosticta sp. (*Euonymus*): **CHINA, Shandong:** campus of Qingdao Agricultural University, in diseased leaves of *Euonymus japonicus* Thunb. (*Celastraceae*), 36°19'16.50"N, 120°24'01.62"E, 9 m, 22 July 2009, MHQAU0248, JJ243.

Phyllosticta sp. (*Portulaca*): **CHINA, Shandong:** campus of Qingdao Agricultural University, as endobiont from stem of *Portulaca oleracea* L. (*Portulacaceae*), 36°19'11.91"N, 120°23'58.18"E, 9 m, 19 July 2008, JJ219.

All the specimens and isolates are deposited in Mycology Herbarium, Qingdao Agricultural University (MHQAU).

Results

Appearance of cultures on PDA

Phyllosticta theacearum: mycelium immersed and superficial, initially pale grey, gradually turning greenish black, dense, floccose, margin undulate with abundant submerged mycelium (Fig. 1A); reverse black (Fig. 1B).

Phyllosticta sp. (*Euonymus*): colonies were initially pale yellow, eventually turning grey with thin submerged mycelium; aerial mycelium scanty, sparse, thin; margin irregular with sparse submerged mycelium (Fig. 1N); reverse with a grey centre surrounded by a black ring and a grey perimeter (Fig. 1O).

Phyllosticta sp. (*Portulaca*): mycelium immersed and superficial, white at first, becoming greyish black; surface covered by a thin mat of whitish aerial mycelium, dense, flat, fissured, with a clearly-defined edge (Fig. 1R); reverse greyish black (Fig. 1S).

Production of pycnidia and conidia on PDA

Phyllosticta sp. (*Portulaca*) produced pycnidia and conidia in 8 days; *Phyllosticta* sp. (*Euonymus*) produced pycnidia and conidia in 7–8 days; *P. theacearum* produced pycnidia and conidia in 14 days.

Loss of appendages with time

Appendages of *P. theacearum*, *Phyllosticta* sp. (*Euonymus*), and *Phyllosticta* sp. (*Portulaca*) disappeared with ageing of colonies on PDA. Conidia of *Phyllosticta* sp. (*Portulaca*) with no visible appendages began to be observed 5 days after conidia were first sighted, and by 8 days no appendages could be seen. Conidia of *Phyllosticta* sp. (*Euonymus*) with no visible appendages began to be observed 3–4 days after conidia were first sighted, and by 6 days no appendages could be seen. Conidia of *P. theacearum* with no visible appendages began to be observed 10 days after conidia were first sighted, and by 15 days no appendages could be seen.

Changes in appearance of appendages in water

Appendages of *Phyllosticta* sp. (*Portulaca*) (Fig. 1T) and *P. cruenta* (Fig. 1G, H) were initially about 50% of the length of conidia. Appendages of *Phyllosticta* sp. (*Euonymus*) (Fig. 1P) and *P. theacearum* (Fig. 1C) were initially between 50% and 100% of the length of conidia. In water mount slides, appendages of *P. cruenta* and *Phyllosticta* sp. (*Euonymus*) extended, while those of *Phyllosticta* sp. (*Portulaca*) (Fig. 1T) and *P. theacearum* (Fig. 1D) remained unchanged, retaining their original length even in water for three days. Appendages of *P. cruenta* began to extend after 6 minutes in water (Fig. 1I) and became 3–4 times the original length in 18–30 min (Fig. 1J, K, arrows). After that, no further extension occurred. In *P. cruenta*, the conidia contained small greenish guttules (Fig. 1G–H) which, with time immersed in water, fused into two large drops (Fig. 1J, K). Guttules in the other three species did not fuse. Appendages

of *Phyllosticta* sp. (*Euonymus*) extended to 2–3 times their original length in 12–30 min (Fig. 1Q, arrow) and no change occurred thereafter.

Discussion

The presence or absence of appendages is routinely used to distinguish *Phyllosticta* species (Nag Raj 1983, Punithalingam & Woodhams 1982, Aa 1973, Weidemann et al. 1982). Aa (1973), however, reported that *Phyllosticta* appendages may be caducous in freshly collected specimens and even in pure cultures, being usually visible only on a proportion of the conidia. For cultures on PDA, the present results confirm that appendages of some species in *Phyllosticta* can disappear with time. Prior to the present study, apparently nothing was known about *Phyllosticta* appendage longevity on artificial media. For all three *Phyllosticta* species studied here on PDA, the longest period of appendage visibility lasted no longer than one month. Earlier reports of *Phyllosticta* species lacking appendages may need to be reviewed in the light of this result. Longevity of appendages in cultures on other artificial media commonly used to grow *Phyllosticta* species also needs to be determined, and it may be advisable to record the age of cultures used when describing *Phyllosticta* appendages.

When mounted in water, appendages of the four species of *Phyllosticta* in the present study remained persistent, but responded differently. Those of *P. theacearum* and *Phyllosticta* sp. (*Portulaca*) kept their original length in water for several days, while those *P. cruenta* and *Phyllosticta* sp. (*Euonymus*) extended 2–4 times their original length in a short time (12–30 minutes). This phenomenon may explain why in earlier reports there is sometimes considerable divergence in the stated length of conidial appendages in the same species. Aa (1973), for example, reported that conidia of *P. vaccinii* were 8–12 × 5–8 µm and had appendages usually 4–8 µm long and sometimes up to 17 µm long, while Weidemann et al. (1982), studying the same species, reported larger measurements of spore appendage lengths. They described appendage lengths as ranging from 3–70 µm for conidia 8–13 × 7–9 µm in size. In another species *P. elongata*, Weidemann et al. (1982) reported appendages varying in length from 4–120 µm. Unfortunately, the time elapsed between preparing the slide and measuring the appendages was not stated. Earlier reports of appendage lengths should therefore be treated with caution. At present it is not clear if this phenomenon of extending appendages is widespread in *Phyllosticta*, or present only in some species. Given its potential impact on *Phyllosticta* identification, more species could usefully be studied.

The identity of the *Phyllosticta* spp. on *Euonymus japonicus* and on *Portulaca oleracea* merits discussion. Numerous taxa described as *Phyllosticta* spp. from *Euonymus* belong in other genera or are of dubious application (Aa & Vanev

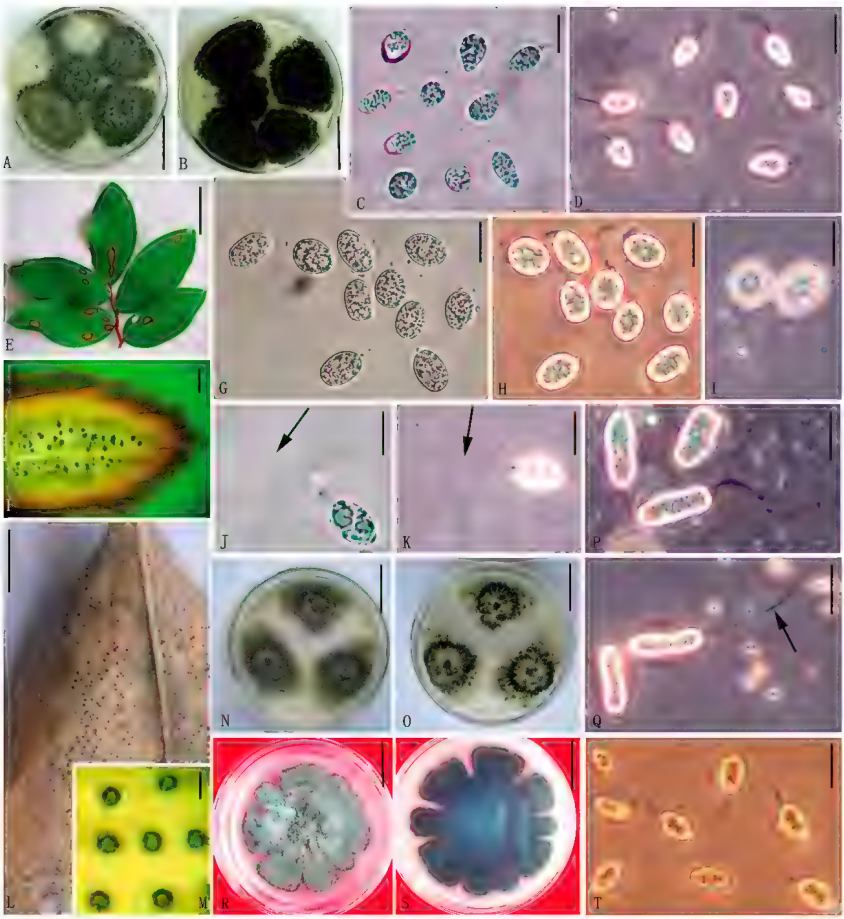

PLATE 1 Four species of *Phyllosticta*. ***Phyllosticta theacearum***: (A) upper colony; (B) converse colony; (C) conidia; (D) conidia after three days in water. ***Phyllosticta cruenta***: (E) symptom on the leaf of *Polygonatum odoratum* var. *pluriflorum*; (F) pycnidia; (G,H) conidia; (I) conidia after 6 min. in water; (J,K) conidia after 18–30 min. in water. ***Phyllosticta* sp.** (on *Euonymus*): (L,M) pycnidia on the leaf of *Euonymus japonicus*; (N) upper colony; (O) converse colony; (P) conidia; (Q) conidia after 12–30 min. in water. ***Phyllosticta* sp.** (on *Portulaca*): (R) upper colony; (S) converse colony; (T) conidia. (C,G,J under light field; D,H,I,K,P,Q,T under phase contrast field.) Scale bars: A,B,E,L,N,O,R,S = 2.5 cm; F = 1 mm; M = 100 μm; C,D,G–K,P,Q,T = 10 μm.

2002). *Phyllosticta euonymi-japonici* L.L. Liu & G.Z. Lu is apparently the only species of *Phyllosticta* sensu stricto described from *Euonymus*; it differs from the present species in shape and dimension of conidia. The conidia of *P. euonymi-japonici* are ellipsoid to ovoid, 10–12.5 × 7.5–10 μm, while those of

Phyllosticta sp. (*Euonymus*) are clavate and 15–23 × 6–8 µm. It therefore seems possible that the present species is undescribed. *Phyllosticta* sp. (*Portulaca*) may also represent an undescribed species. There is no named species of *Phyllosticta* recorded on *Portulaca*, but Batista & Vital (1952: 57) reported *Phyllosticta* sp. on *P. oleracea* from Brazil. There is insufficient information to determine whether the Brazilian fungus is a species of *Phyllosticta* sensu stricto, or to compare it with our *Portulaca* isolate.

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Notes on some Japanese smut fungi. 5. *Anthracoidea blepharicarpae* and *A. dispalatae,* spp. nov.

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Abstract — Two new smut fungi, *Anthracoidea blepharicarpae* on *Carex blepharicarpa* and *A. dispalatae* on *Carex dispalata,* are described and illustrated from Japan.

Key words — *Anthracoideaceae,* taxonomy, *Ustilaginomycetes*

Introduction

During a taxonomic revision of the genus *Anthracoidea* in Japan, we found a specimen of a verruculose-spored species with relatively large spores on *Carex blepharicarpa.* After comparison with the known species of *Anthracoidea* on *Carex* sect. *Mitratae,* we consider this fungus new. The host of another Japanese specimen, originally identified as *Anthracoidea caricis* on *Carex* sp., was found to represent *Carex dispalata,* which is a member of the sect. *Anomalae.* Species of *Anthracoidea* have been considered by Vánky (1979) to be restricted to host plants belonging to the same or closely related sections of *Carex.* As no species of *Anthracoidea* has previously been reported on a representative of *Carex* sect. *Anomalae,* we propose a new species for this collection.

Material & methods

Material from the mycological collections the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba (TSH), and Faculty of Agriculture, Hokkaido University, Sapporo (SAPA), was examined under the light (LM) and scanning electron (SEM) microscopes. For LM observations, spores were mounted in lactophenol solution on glass slides, gently heated to boiling point, and then cooled.

Spore measurements are given in the form: min–max (mean ± 1 standard deviation). For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with gold with an ion sputter. The surface structure of spores was observed at 10 kV and photographed with a JEOL SM-6390 scanning electron microscope.

Taxonomy

Anthracoidea blepharicarpae Denchev, T. Denchev & Kakish., **sp. nov.** FIGS 1–2

MycoBank MB 519311

Sori in ovariis in inflorescentia dispersi, sicut corpora ellipsoidea vel basi cuneata, nigra, 3–4 mm longa, in superficie pulverei. Sporae a fronte visus late ellipticae, irregulares vel angulares, 18–30 × 14–25 (24.4±2.1 × 20.7±1.8) µm, a latere visus 12.5–15 µm, rufobrunneae vel atro-rufobrunneae; paries inaequaliter incrassatus, 1–3 µm crassus, plerumque 1–3 (–5) gibberis internis, et interdum etiam maculis lucem refringentibus; superficie verruculosa.

***Holotypus** in matrice* Carex blepharicarpa *Franch.: JAPONIA, Hokkaido, Otaru (Ishikari Prov.), Mt. Tengu, 19.VI.1932, leg. Otani, Yabe, Takee & Murayama (SAPA, sine num.).*

Etymology: the name refers to the host species.

Sori in ovaries, scattered in the inflorescence, as ellipsoidal or cuneate at base, black, hard bodies, 3–4 mm long, when young covered by a thin membrane, later becoming exposed; spore mass of the mature sori powdery on the surface. Spores flattened, in plane view broadly elliptical, irregular or moderately angular in outline, sometimes elliptical or oval, in plane view 18–30 × 14–25 (24.4±2.1 × 20.7±1.8) µm (n = 150), as an exception up to 34 µm long, in side view 12.5–15 µm thick, middle to dark reddish brown, wall unevenly thickened, 1–3 µm thick, thickest at the angles, with 1–3 (–5) internal swellings, sometimes with light-refractive spots; verruculose. Spore germination unknown.

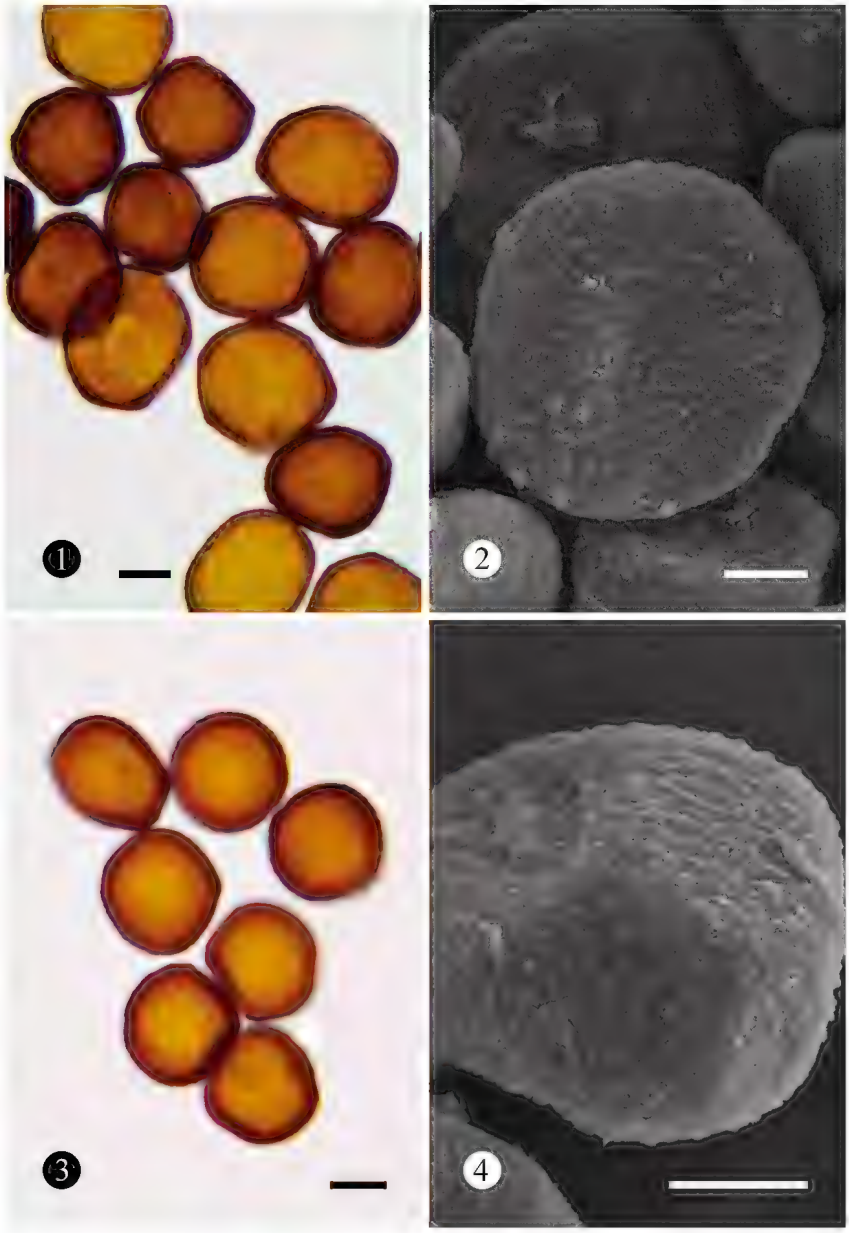
Distribution — On *Cyperaceae: Carex* – subgen. *Carex*, sect. *Mitratae: C. blepharicarpa*, Asia (Japan – Hokkaido).

Comments — *Carex blepharicarpa* is an eastern Asian species distributed in Japan, Korea, Kuril Islands, and Sakhalin Island.

Anthracoidea blepharicarpae differs from the two other members of *Anthracoidea* on sedges of sect. *Mitratae*, *A. microsora* (Syd.) Kukkonen and *A. caryophylleae* Kukkonen, in having larger spores.

The mean values of the spore length of 17 Japanese specimens of *A. microsora* as measured by us fell into a range of 18.8–20.7 µm. The mean value of the spore length of these specimens was 19.6±1.7 µm.

The mean values of the spore length of 18 eastern Asian specimens of *A. caryophylleae* (14 Japanese, two Korean, and two from the Sakhalin Island) as measured by us fell into a range of 18.6–21.7 µm. The mean value of the spore length of these specimens was 20.4±1.7 µm. Both mean values of the spore length of *A. microsora* and *A. caryophylleae* differed significantly from the respective mean of 24.4±2.1 µm for *A. blepharicarpae*.



Figs 1–2. Spores of *Anthracoidea blepharicarpae* on *Carex blepharicarpa* in LM and SEM (Holotype). Figs 3–4. Spores of *Anthracoidea dispalatae* on *Carex dispalata* in LM and SEM (Holotype). Scale bars: 1, 3 – 10 μm; 2, 4 – 5 μm.

Anthracoidea caryophylleae is a species originally described from Europe (Finland, Kukkonen 1963), where it is a common species (especially in Central and East Europe, and the Balkan Peninsula). The mean values of the spore length of European specimens were in a range of 16.9–20.5 µm (Kukkonen 1963: 18, Nannfeldt 1979, Vánky 1979: 229, Scholz & Scholz 1988, Denchev 2001) (cfr with the above noted range of 18.6–21.7 µm). It seems that the spores of the European specimens of *A. caryophylleae* are shorter than those of the eastern Asian specimens.

Key to the *Anthracoidea* species on subgen. *Carex* sect. *Mitratae*

1 Spores 18–30 (–34) µm long, spore mean length more than 22.5 µm ..... ***A. blepharicarpae***

1* Spores 15–25 (–27.5) µm long, spore mean length less than 22.0 µm 2

2 Spores finely verruculose, warts up to 0.3 µm high; profile smooth or nearly so ..... ***A. caryophylleae***

2* Spores verruculose to verrucose, warts 0.2–0.6 µm high, affecting the spore profile ..... ***A. microsora***

Anthracoidea dispalatae Denchev, T. Denchev & Kakish., **sp. nov.** FIGS 3–4

MYCOBANK MB 519312

SORI in ovariis in inflorescentia dispersi, sicut corpora subglobosa vel ovoidea, nigra, 1.8 2 mm longa, in superficie pulverei. SPORAE a fronte visus irregulares, interdum suborbiculares vel late ellipticae, 19 26 × 16.5 23 (22.3 ± 1.5 × 19.3 ± 1.5) µm, a latere visus 11.5 14 µm, rufobrunneae vel atro-rufobrunneae; paries inaequaliter incrassatus, 1.5 2.5 (3.3) µm crassus, plerumque sine gibberis internis, interdum 1 gibba interna, sine maculis lucem refringentibus; superficie verruculosa.

***Holotypus** in matrice* Carex dispalata *Boott ex A. Gray: JAPONIA, Honshu, Niigata Pref., Gosen-shi (Nakakanbara-gun), 2.VI.1912, leg. K. Yoshino (TSH, sine num.).*

ETYMOLOGY: the name refers to the host species.

SORI in ovaries, scattered in the inflorescence, as subglobose or ovoid, black, hard bodies, 1.8–2 mm long, when young covered by a thin membrane, later becoming exposed; spore mass of the mature sori powdery on the surface. SPORES flattened, in plane view moderately irregular, sometimes suborbicular or broadly elliptical in outline, in plane view 19–26 × 16.5–23 (22.3±1.5 × 19.3±1.5) µm (n = 150), in side view 11.5–14 µm thick, middle to dark reddish brown, wall slightly unevenly thickened, 1.5–2.5 (–3.3) µm thick, without internal swellings, sometimes with one, without light-refractive spots; verruculose. In SEM warts often forming small groups or short rows; the wall between the warts finely and irregularly punctate. SPORE GERMINATION unknown.

DISTRIBUTION — On *Cyperaceae: Carex* – subgen. *Carex,* sect. *Anomalae: Carex dispalata,* Asia (Japan – Honshu).

COMMENTS — Based on Egorova (1999), *Carex dispalata* is a representative of sect. *Anomalae* sensu lato, which includes some smaller sections, described by Japanese authors (e.g., *Glaucaeformes* Ohwi, *Molliculae* Ohwi, *Confertiflorae* Franch. ex Ohwi, *Dispalatae* Ohwi, *Alliiformes* Akiyama). In a recently published monograph of China (Dai et al. 2010), *Carex dispalata* is included in sect. *Confertiflorae*. *Carex dispalata* is distributed in the Far East of Russia, Sakhalin Island, south Kuril Islands, Japan, Korea, and NE and Central China.

Acknowledgements

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Notes on two species of *Boletellus* (*Boletaceae, Boletales*) from China

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ABSTRACT Based on studies of the types and additional materials, *Boletellus emodensis* and *B. obscurecoccineus* are described and illustrated in detail. *Strobilomyces annamiticus* is lectotypified and synonymized with *B. emodensis*; specimens from China regarded as *B. ananiceps* are confirmed to represent *B. emodensis*; *B. puniceus* and *Boletus megasporus*, originally described from southwestern China, are conspecific with *B. obscurecoccineus*.

KEY WORDS morphology, revision, taxonomy, type studies

Introduction

Species of *Boletellus* Murrill are widely distributed and highly diverse in China, and about twenty-five taxa have been reported from the country (Chiu 1948, 1957; Teng 1963; Bi et al. 1982, 1984, 1997; Wen 1985; Zang 1985; Zang & Yuan 1999; Zang et al. 1999; Li & Song 2003). Recently, some field and laboratory observations on the species of genus *Boletellus* from southern and southwestern China were made, and several herbarial specimens including the types of two species were re-examined. The field observations and critical re-examinations indicate that some purported species of the genus are later synonyms. Thus, our new findings update characteristics and distributions for *B. emodensis* and *B. obscurecoccineus*.

Materials & methods

Basidiomata were collected during the rainy season (April–September) in southern and southwestern China. Specimens were described and/or photographed in the field, dried, and then deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). Additional collections from the

Farlow Herbarium, Harvard University (FH), and the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM) were examined. 5% KOH was used as a mounting medium for microscopic studies. The notations "basidiospores (n/m/p)" indicate that the measurements were made on n basidiospores from m basidiomata of p collections. Dimensions of basidiospores including the ornamentation are given using notation of the form (a) b-c (d); the range b-c contains a minimum of 90% of the measured values, and extreme values a and d are given in parentheses. Q refers to the length/breadth ratio of basidiospores; Q_m refers to the average Q of basidiospores ± sample standard deviation. All line drawings of microstructures were made from rehydrated material.

Taxonomy

Boletellus emodensis (Berk.) Singer, Annls mycol. 40: 19, 1942. FIGS. 1–11

≡ *Boletus emodensis* Berk., Hook. J. Bot. Kew Gard. Misc. 3: 48, 1851.

Strobilomyces annamiticus Pat., Bull. Soc. Mycol. Fr. 25: 6, 1909.

≡ *Boletellus annamiticus* (Pat.) E.-J. Gilbert, Les Bolets: 107, 1931.

[The description below is based on HKAS 59211, HKAS 59213, GDGM 13229, HKAS 59214, HKAS 59212, HKAS 37832, and HKAS 54741.]

MACROCHARACTERS — BASIDIOMATA medium-sized. PILEUS 4.5–9 cm in diameter, convex to applanate; surface dry, purple to dull crimson, usually fading to pale fawn in age, finely tomentose when young, then cracking into large squamules; margin at first extended into a false veil and covering the pores, then splitting radially, appendiculate with false veil remnants; context 0.8–1.1 cm thick in the center of pileus, yellowish, cyanescent strongly and rapidly when injured. HYMENOPHORE poroid, yellow, cyanescent strongly and rapidly when injured, depressed around apex of stipe; pores 0.1–0.2 cm in diameter, simple, angular. STIPE central, 6–8 × 0.8–1 cm, cylindric, solid, firm; surface dry, fibrous, concolorous with the pileus, but apex yellowish; base usually enlarged and villous with white mycelium; context yellow, cyanescent strongly and rapidly when injured. ANNULUS absent (FIGS. 1–5).

MICROCHARACTERS — BASIDIA 34–52 × 11–17 µm, clavate, colorless to yellowish brown in KOH, 4-spored (occasionally 2- or 3-spored) (FIG. 6). BASIDIOSPORES [260/13/7] (16–)18–23(–25) × (7–)8–10(–12) µm, Q = (1.70–)1.89–2.75(–2.87), Q_m = 2.28 ± 0.24, yellowish to yellowish brown in KOH, elongate-subfusoid to elongate-ellipsoid, with longitudinal or oblique ridges, 7–9 ridges visible in lateral view; ridges up to 1–1.5 µm in height, round in transverse section, having distinct transverse striations on the ridges (FIGS. 7–9). TUBE TRAMA made up of thin-walled, colourless to yellowish hyphae 5–13 µm in width. CHEILOCYSTIDIA 48–66 × 11–16 µm, subfusiform to fusiform, thin-walled, colorless or yellowish to yellowish brown in KOH (FIG. 10). PLEUROCYSTIDIA 70–90 × 11–18 µm, subfusiform to fusiform, colorless to yellowish brown in KOH (FIG. 6). PILEIPELLIS an intricate trichoderm composed of interwoven,

FIGS. 1–2: Basidiomata of *Boletellus emodensis* (HKAS 59212). 1. Immature basidioma (HKAS 59215). 2. Fruiting habit on tree stump in forest dominated by *Fagaceae*, Fujian, China. Bars = 2 cm.

thin-walled, yellowish to yellowish brown hyphae; terminal cells 19–35 × 7–11 µm, with round to obtuse apex (FIG. 11). PILEAL TRAMA made up of thin-walled, colorless to yellowish hyphae 5–15 µm in diameter. CLAMPS absent in all tissues.

HABIT, HABITAT, DISTRIBUTION — Solitary or gregarious on the ground, tree stumps or rotten wood in forests dominated by *Fagaceae*; originally described from India; then reported from southeast Asia (Corner 1972, Horak 1977), east Asia (Hongo & Izawa 1994, Chen et al. 1997, Mao 2009, An 1998), and Australia (Young 2004).

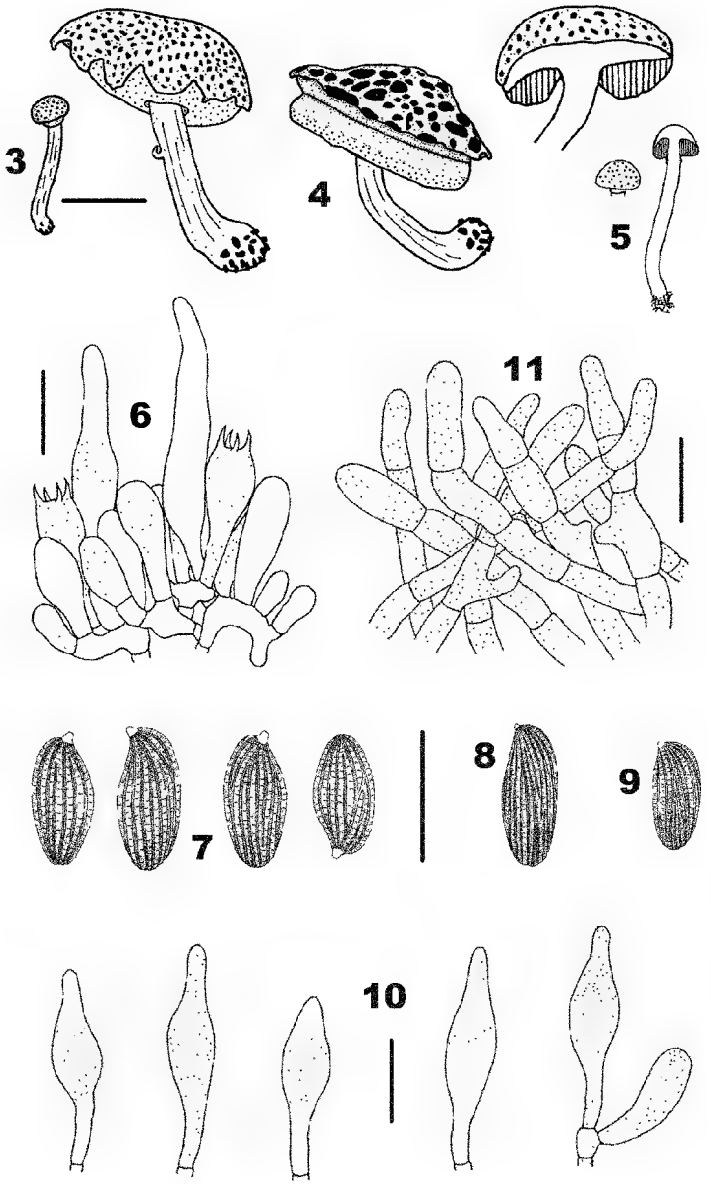
SPECIMENS EXAMINED — **VIETNAM. Tonkin**: Rives du Kam-ly, 1907, det. N.T. Patouillard, Eberhardt LBA 161 (FH 3720 pro parte, ***Strobilomyces annamiticus* lectotype designated here**); Vallée du Da-Pounian, 1907, det. N.T. Patouillard, Eberhardt LBA 209 (FH 3720 pro parte, *Strobilomyces annamiticus*). **CHINA. Hainan Province**: Baisha County, Yinggeling Mountain, alt. 747 m, 28 July 2009, N.K. Zeng 384 (HKAS 59211); same location and date, alt. 841 m, N.K. Zeng 379 (HKAS 59215); Qiongzhong County, Limushan Mountain, alt. 869 m, 4 August 2010, N.K. Zeng 829 (HKAS 59213); Ledong County, Jianfengling Mountain, 4 October 1987, T.H. Li s. n. (GDGM 13229, as "*B. ananaeceps*" in Bi et al. 1997); **Guangdong Province**: Zhaoqing County, Dinghushan Mountain, alt. 233 m, 9 August 2010, N.K. Zeng 867 (HKAS 59214); **Fujian Province**: Zhangping County, Xinqiao Town, Chengkou Village, alt. 357 m, 3 September 2009, N.K. Zeng 674 (HKAS 59212); **Yunnan Province**: Jingdong County, Qincaitang, 15 September 1999, Q.B. Wang 282 (HKAS 37832); Jiangcheng County, alt. 1400 m, 29 July 2008, L.P. Tang 510 (HKAS 54741).

COMMENTS — *Boletellus emodensis* is well characterized by its purple to dull crimson pileus, a yellow context in both pileus and stipe that is strongly and rapidly cyanescent in both when injured, longitudinally ridged basidiospores with fine cross-striations on the ridges observable with the light microscope. Although *B. emodensis* is quite common throughout east and southeast Asia, it was not well characterized until the publication by Corner (1972).

Strobilomyces annamiticus was described based on collections from Vietnam (Patouillard 1909) and later transferred to *Boletellus* by Gilbert (1931). One of us (ZLY) studied the syntypes of *S. annamiticus* deposited in Farlow Herbarium, Harvard University (FH 3720), which consist of two collections, both identified as *S. annamiticus* by N.T. Patouillard. One collection (Eberhardt LBA 161) is composed of a single fruitbody (Fig. 5). The dried cap is about 1 cm in diameter, convex, dark chocolate brown, covered with dull chocolate brown tomentose squamules, and the pileal margin is appendiculate. The stipe is 7 × 0.3 cm, with the base apparently attached to wood. The basidiospores (Fig. 8) are [20/1/1] (20–)21–24(–26) × (6.5–)7–8.5 μm, Q = 2.63–3.29(–3.69), Q_m = 2.95 ± 0.30, yellowish to yellowish brown in KOH, elongate-subfusoid to elongate-ellipsoid, with longitudinal or oblique ridges, 7–9 ridges visible in lateral view, with ridges up to 1 μm in height, having indistinct transverse striations on the ridges. The other collection (Eberhardt LBA 209) consists of a dried cap which is 1.5–2 cm in diameter, convex to applanate, dark to blackish. The stipe is 5 × 0.2–0.4 cm, on rotten wood. The basidiospores (Fig. 9) are [20/1/1] (15–)16–18 × (6.5–)7.5–8.5 μm, Q = (1.76–)1.88–2.33(–2.46), Q_m = 2.10 ± 0.16, yellowish brown in KOH, elongate-subfusoid to elongate-ellipsoid, with longitudinal or oblique ridges, 7–9 ridges visible in lateral view, with ridges up to 1 (1.5) μm in height, round in transverse section, having distinct transverse striations on ridges.

In the protologue of *Strobilomyces annamiticus*, Patouillard (1909) cited two localities that correspond exactly with the localities of the collections Eberhardt LBA 161 and Eberhardt LBA 209. Eberhardt LBA 161 is designated here as lectotype. Singer (1945) regarded the two Vietnamese collections as immature basidiomata of *B. emodensis*, and *S. annamiticus* was synonymized with *B. emodensis*. With our re-examination of the syntypes and field observation on other collections, the present authors are convinced that the basidiomata of the two Vietnamese collections are the same as immature basidiomata of *B. emodensis*, because the size of basidiospores of *B. emodensis* may be variable at different stages of basidioma development. For example, the basidiospores of one immature basidioma (HKAS 59215) are [20/1/1] 15–18(–19) × 5–7 μm, with Q = (2.14–)2.29–3.17(–3.20) and Q_m = 2.73 ± 0.28. They are clearly much shorter and narrower when compared to mature collections. This explains why the basidiospores from the two Vietnamese collections are slightly narrower (and Eberhardt LBA 209 slightly shorter) compared with mature Chinese material and the holotype (20–24 × 8–10 μm) (Pegler & Young 1971: 163) of *B. emodensis*, and further confirms that *S. annamiticus* is a synonym of *B. emodensis*, as noted by Singer (1945).

Boletellus ananiceps (Berk.) Singer, originally described from Australia, is allied to *B. emodensis*. However, *B. ananiceps* has been noted to differ from



Figs. 3–11: *Boletellus emodensis* (= *B. annamiticus*) [3 from HKAS 59215; 4 from HKAS 59212; 5, 8 from Eberhardt LBA 161 of FH 3720 (*B. annamiticus*); 6, 7, 10, 11 from HKAS 59211; 9 from Eberhardt LBA 209 of FH 3720 (*B. annamiticus*)]. 3–5. Basidiomata. 6. Basidia and pleurocystidia. 7–9. Basidiospores. 10. Cheilocystidia. 11. Pileipellis. Bars: 3–5 = 2 cm, 6–11 = 20 μm.

B. emodensis by its longitudinally ridged basidiospores that lack cross-striations on the ridges (Singer 1955, Bougher & Syme 1998, Halling & Fechner 2011). In China, *B. ananiceps* was previously reported from Hainan (Bi et al. 1997) with three cited collections (GDGM 13229, GDGM 16785, GDGM 15741). Unfortunately, the voucher specimens GDGM 16785 and 15741 were lost (Ms. Z.D. Xiao, personal communication). Re-examinations of GDGM 13229 showed that that collection is identical to *B. emodensis* in its purple to dull crimson pileus and obvious cross-striations on the basidiospore ridges. Therefore, whether *B. ananiceps* occurs in China remains to be confirmed.

Boletellus ananas (M.A. Curtis) Murrill, a species originally described from USA, is also allied with *B. emodensis*. However, the pileus of the former is pink or red when fresh and becomes pale fuscous tan when it fades; the stipe is never red, as in *B. emodensis*; the squamae in *B. ananas* are coarse, while in *B. emodensis* usually fine. Furthermore, the ridges on the basidiospores are broader than those of *B. emodensis* (Halling 2010). In China, *B. ananas* was previously reported from the south of China (Chiu 1948, 1957; Teng 1963; Zang 1985; Bi et al. 1994). Judging from their descriptions, these reports may represent *B. emodensis*. Thus, whether *B. ananas* occurs in China also needs confirmation.

Boletellus obscurecoccineus (Höhn.) Singer, Farlowia 2:127, 1945. FIGS. 12–22

≡ *Boletus obscurecoccineus* Höhn., Sber. K. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1, 123: 88, 1914.

Boletus puniceus W.F. Chiu, Mycologia 40(2): 217, 1948.

≡ *Xerocomus puniceus* (W.F. Chiu) F.L. Tai, Syll. Fung. Sin.: 815, 1979.

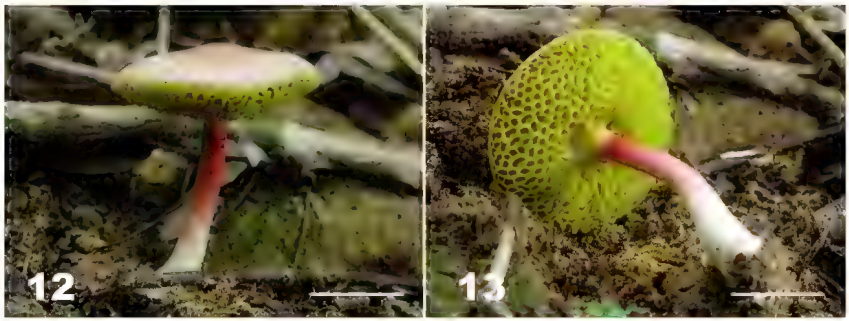
≡ *Boletellus puniceus* (W.F. Chiu) X.H. Wang & P.G. Liu, Mycotaxon 84: 128, 2002.

Boletus megasporus M. Zang, Acta Microbiol. Sin. 20(1): 30, 1980, as "*magasporus*".

[The description below is based on FH 2993, HKAS 59208, HKAS 59209, and HKAS 59210.]

MACROCHARACTERS — BASIDIOMATA small to medium-sized. PILEUS 1.5–7 cm in diameter, convex to applanate; surface dry, pink to pale crimson when fresh, becoming dark purplish red to dark purple when dried, finely tomentose when young, then minutely cracked; context yellowish, unchanging in color when injured. HYMENOPHORE poroid, yellow, unchanging in color when injured, depressed around apex of stipe; pores 0.1– 0.2 cm in diameter, simple, angular. STIPE central, 3–5 × 0.2–0.8 cm, cylindric, slightly attenuate upwards, solid, firm, apex yellowish, middle finely tomentose when young, then fracturing into pale crimson squamules, with base usually enlarged and villous with white mycelium; context whitish, unchanging in color when injured. ANNULUS absent (FIGS. 12–16).

MICROCHARACTERS — BASIDIA 42–55 × 8–12 μm, clavate, colorless to yellowish in KOH, 4-spored (sometimes 2- or 3-spored) (FIG. 17).



Figs. 12–13: Basidiomata of *Boletellus obscurecoccineus* (HKAS 59208). 12. Fruiting habit on soil in forest dominated by *Lithocarpus*, Hainan, China. 13. Ventral. Bars = 2 cm.

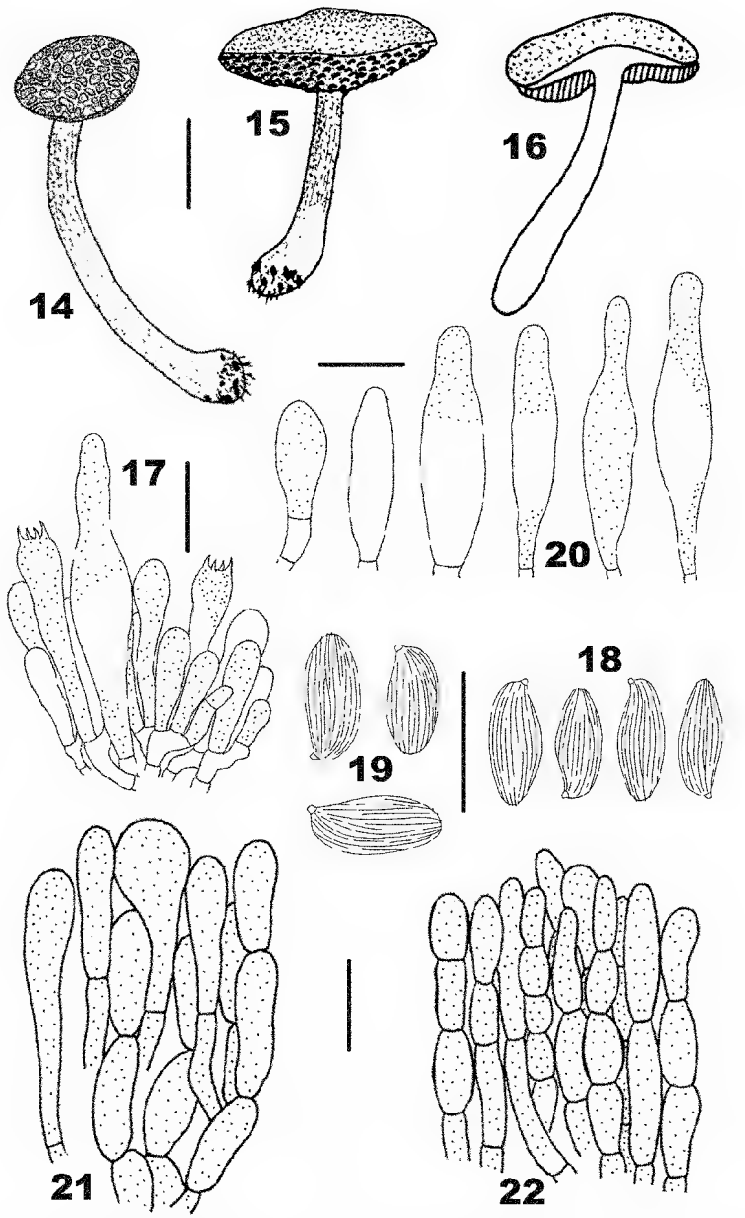
Basidiospores [140/7/4] (14–)14.5–18(–20.5) × (5–)6–8(–9.5) µm, Q = (1.81–)2.00–2.77(–3.30), Q_m = 2.32 ± 0.26, brownish to pale ochraceous in KOH, elongate-subfusoid to elongate-ellipsoid, with 7–10 faintly longitudinal ridges in lateral view, not transversely striate on ridges (Figs. 18–19). Tube trama made up of thin-walled hyphae 4–9 µm in width, colorless to yellowish in KOH. Cheilocystidia 32–87 × 11–17 µm, subfusiform to fusiform, thin-walled, colorless to yellowish in KOH (Fig. 20). Pleurocystidia similar to cheilocystidia (Fig. 17). Pileipellis an epithelioid trichoderm made up of vertically arranged, ellipsoid, occasionally sphaeropedunculate, thin-walled (sometimes up to 1 µm in thickness), yellowish to yellowish brown hyphae; terminal cells 22–54(–90) × 9–15(–60) µm, with round to obtuse apex (Figs. 21–22). Pileal trama made up of thin-walled, colorless to yellowish hyphae 6–12 µm in diameter. Clamps absent in all tissues.

Habit, habitat, distribution — Solitary or scattered on the ground in forests of *Lithocarpus* (*Fagaceae*), occasionally on rotten wood; originally described from Indonesia (Höhnel 1914: 88), and later reported from east Asia (Hongo 1970, Wen 1985, Chen et al. 1997, An 1998, Wang & Liu 2002, Yuan & Sun 2007), southeast Asia (Corner 1972, Horak 1977) and Australia (May & Wood 1997, Bougher & Syme 1998).

Specimens examined — **Indonesia. Java**: Tjibodas, 1908, leg. F.X.R. v. Höhnel, *Boletus* 1187 (FH 2993, holotype). **China. Hainan Province**: Baisha County, Yinggeling Mountain, alt. 943 m, 26 July 2009, N.K. Zeng 328 (HKAS 59208); same location and date, alt. 968 m, N.K. Zeng 335 (HKAS 59209); same location, alt. 824 m, 28 July 2009, N.K. Zeng 367 (HKAS 59210).

Comments — *Boletellus obscurecoccineus* is well characterized by its pink to pale crimson pileus, faintly longitudinally ridged basidiospores combined with the broad pores, and non-cyanescent context.

One of us (ZYL) studied the holotype of *B. obscurecoccineus*, which consists of two and a half basidiomata. The dried cap is about 3–5 cm in diameter, the



Figs. 14–22: *Boletellus obscurecoccineus* (14, 17, 20, 22 from HKAS 59210; 15, 18 from HKAS 59208; 16, 19, 21 from FH 2993, holotype).14 16. Basidiomata. 17. Basidia and pleurocystidium. 18 19. Basidiospores. 20. Cheilocystidia. 21 22. Pileipellis. Bars: 14 16 2 cm, 17 22 20 μm.

pileal surface is finely tomentose and dark purplish red to dark purple when dried, the hymenophore is umber-brown, with pores about 1 mm in diameter, the stipe (4–5 × 0.4–0.6 cm) attenuates slightly upwards, and the surface is finely tomentose, whitish to ochraceous, much paler than cap (Fig. 16). Basidia and cystidia would not rehydrate sufficiently for study. Basidiospores (Fig. 19) are [21/1/1] (15–)16–20.5 × 7.5–8.5(–9.5) µm, Q = 2.00–2.38(–2.47), Q_m = 2.20 ± 0.13, brownish to pale ochraceous in KOH, elongate-subfusoid to elongate-ellipsoid, with 7–10 faint longitudinally ridges in lateral view, without transverse striations on ridges. The pileipellis (Fig. 21) is an epithelioid trichoderm made up of vertically arranged, moniliform hyphae with ellipsoid, occasionally sphaeropedunculate, thin-walled elements, often with yellowish-brownish vacuolar pigment; terminal cells are 25–50(–90) × 10–15(–60) µm, with round to obtuse apex.

Boletus puniceus was originally described from Yunnan, China (Chiu 1948, 1957). Without examining the type of *B. puniceus*, Corner (1972) suspected *B. puniceus* was conspecific with *B. obscurecoccineus*. Wang & Liu (2002) re-examined the type of *B. puniceus* (HMAS3852) and found the basidiospores to be [74/1/1] 15–18(–19.3) × (6.3–)6.7–8(–9) µm, Q = (1.90–)2.05–2.54(–2.64), Q_m = 2.30 ± 0.16, with faint longitudinal ridges, and the species was transferred to *Boletellus*, with *Boletus megasporus* included as a synonym. The present type studies showed no essential differences among *B. obscurecoccineus*, *B. megasporus* and *B. puniceus*, suggesting that they are conspecific.

Mao (2000: Fig. 830) published a photo labelled as *Aureoboletus* [as "*Austreoboletus*"] *thibetanus* (Pat.) Hongo & Nagas. Judging from the features of the basidiomata in the photo, it may represent *B. obscurecoccineus*, as previously noted by Yang et al. (2003).

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Aspergillus flavus — primary causative agent of aflatoxins in dried figs

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Abstract — *Aspergillus* sect. *Flavi* isolates from Turkish dried figs have been determined using PCR amplification of the ITS1 and ITS2 rDNA regions. Species were identified by comparing partial 18S rDNA sequences from 57 different fungal isolates comprising 5 *A. parasiticus* strains, 1 *A. tamarii* strain, and 51 *A. flavus* strains with known ribosomal sequences using BLAST search. Sequence comparisons between the isolates and reference cultures showed a 95 99% similarity; morphological and phenotypical character comparisons of the same strains produced equally close similarities. The sole exception was an aflatoxin B_1 and B_2 producing strain that cannot produce cyclopiazonic acid; this strain, originally identified as *A. flavus*, was instead found to represent *A. parasiticus*.

Key words *Aspergillus parasiticus*, Polymerase Chain Reaction, dried fruit, ITS, CPA

Introduction

The *Aspergillus flavus* group commonly found in nature is one of the most widely studied fungal species. *Aspergillus flavus, A. parasiticus, A. nomius, A. pseudotamarii,* and *A. bombycis* produce aflatoxin, a potent carcinogenic toxin, while *A. oryzae, A. sojae,* and *A. tamarii* are non-toxigenic species widely used in the production of fermentation products throughout Asia (Frisvad et al. 2007). Although these fungi are commonly used to produce enzymes and fermentation products, some cause serious problems through contamination of food and foodstuffs.

Aflatoxins (AFs), naturally occurring secondary metabolites, are potent hepatotoxic, mutagenic, and carcinogenic toxins causing serious health hazards

in humans and animals. Aflatoxin B_1, B_2, G_1 and G_2 are found predominantly in food whereas M_1 and M_2 aflatoxins are found primarily in animal tissues and fluids (milk, urine) as the hydroxylated metabolic products of aflatoxins B_1 (AFB_1) and B_2 (AFB_2) respectively (Richard 2007). Special interest is given to AFs due to their high occurrence and toxicity. The most toxic aflatoxin known, AFB_1, is cited as a group I carcinogen by the International Agency for Research on Cancer (IARC 1993). The maximum levels of AFB_1 and total aflatoxins allowable in dried fruits as determined by Commission of the European Communities are 2 and 4μg/kg respectively. Aflatoxigenic fungi occur in such food commodities as corn, peanuts, cottonseed, nuts, spices, figs, and dried fruits (Pitt & Hocking 1997). Aflatoxigenic fungi may contaminate foods throughout several stages (pre-harvest, processing, transportation, storage) of the food chain (Manonmani et al. 2005). The level of mould infestation and identification of species are important indicators of raw material quality and predictors of the potential risk of mycotoxin occurrence (Shapira et al. 1996).

Fungi in foods can be identified using traditional morphological and phenotypic observations of cultures in different media. However, such methods are time consuming and laborious and require mycological expertise and special facilities. Also, because morphological characters can vary over time, they should be verified by the mycotoxigenic profile of the strains (Samson et al. 2007). Furthermore, traditional methods do not distinguish toxigenic from non-toxigenic forms. For these reasons, application of molecular techniques has become increasingly important in detecting and characterizing potential contaminants within the food-processing industry. These methods are very rapid, specific, and sensitive compared to traditional methods.

There are two approaches in identifying aflatoxigenic fungi through PCR-based methodology. The first uses standard PCR to detect aflatoxigenic fungi in foods. In this technique, the contiguous Internal Transcribed Spacer (ITS) regions in fungal DNA fungi are amplified, sequenced, and compared extensively with sequences in GenBank (Hinrikson et al. 2005; Rodrigues et al. 2007; González-Salgado et al. 2008). The second approach is especially useful for the strains having nearly identical genes, as the PCR reaction targets six aflatoxin biosynthesis genes, namely; aflatoxin regulatory genes (AFLR, AFLS), norsolorinic acid reductase (NOR-1 = AFLD), versicolorin A dehydrogenase (VER-1) sterigmatocystin-o-methyltransferase (OMT-A), polyketide synthase (PKSA), and APA-2 in *A. flavus* and *A. parasiticus* (Shapira et al. 1996; Somashekar et al. 2004; Manonmani et al. 2005; Rodrigues et al. 2009). We used the first approach to detect ITS sequences from 57 *Aspergillus* sect. *Flavi* isolates, which had been previously characterized according to their morphological and phenotypic characters, from Turkish dried figs. This is the first report of molecular analyses of *Aspergillus* sect. *Flavi* strains isolated from a Turkish agricultural commodity.

Materials & methods

Fungal isolates and culture conditions

Fifty-seven strains of *A. flavus*, *A. parasiticus,* and *A. tamarii,* isolated from dried figs from the west of Turkey (Aegean region), were selected. The isolates had been previously morphologically and phenotypically identified to species level (Heperkan & Karbancıoglu-Güler 2009). All fungal cultures were maintained on slant malt extract agar (MEA) and stored at 4°C. Incubation was performed at 25°C for 5 days. Cultures were inoculated with 1 cm diameter mycelial disks cut from the plates and incubated in 30 ml Erlenmeyer flasks containing 20 ml of Potato Dextrose Broth (PDB) at 25°C and 150 rpm. Mycelia from 2-day-old cultures were harvested by filtration through Whatman No.1 paper and kept at –80°C for DNA isolation.

Fungal DNA extraction

Template DNA was extracted from 50–100 mg (wet weight) of fungal mycelia harvested from freshly growing cultures in PDB under stationary conditions. DNA was isolated by using ZR fungal / bacterial DNA system (purchased from Zymo Research, Orange, CA, USA) according to the manufacturer's protocol.

PCR amplification

DNA was amplified by PCR using fungal-specific 18S rDNA primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR amplification protocol was as follows: 1 cycle of 95°C for 5 min, 30 cycles of a set including 95°C for 1 min, 55°C for 1 min and 72°C for 1 min 40 s and a final cycle of 72°C for 10 min. Amplification of fungal DNA was carried out in a total reaction volume of 50 µl, containing 1 µl of template DNA, 1 µl of each primer, 5 µl of 10 x PCR buffer, 4 µl of $MgCl_2$ (25 mM), 1 µl of dNTPs and 0.5 µl of Taq DNA polymerase. The PCR reaction was performed using Biometra T Professional Thermocycler (Biometra, Goettingen, Germany).

DNA sequencing & analysis

Sequencing was carried out using an ABI PRISM 3100-Avant automated sequencer at the Molecular Biology and Genetics Department, ITU. Both strands of the PCR product were sequenced with the same primers. The resultant nucleotide sequences were aligned with MEGA sequence analysis software version 4.0 (Tamura et al. 2007).

Species were identified by comparing the partial 18S rDNA sequences with known ribosomal sequences using BLAST (Basic Local Alignment Search Tool). Comparisons of reference strain and isolate sequences were performed using Clustal W alignment algorithm. Sequence variation and similarity between sampled sequences were determined with MEGA 4.0 software; sequences were visually confirmed using pairwise nucleotide alignments.

Results & discussion

ITS regions from 57 *Aspergillus* sect. *Flavi* strains were sequenced to identify the species at the molecular level. ITS regions, located between the 18S and 28S RNA genes, offer distinct advantages over other molecular regions due to the existence of approximately 100 copies per genome. Amplification of the ITS

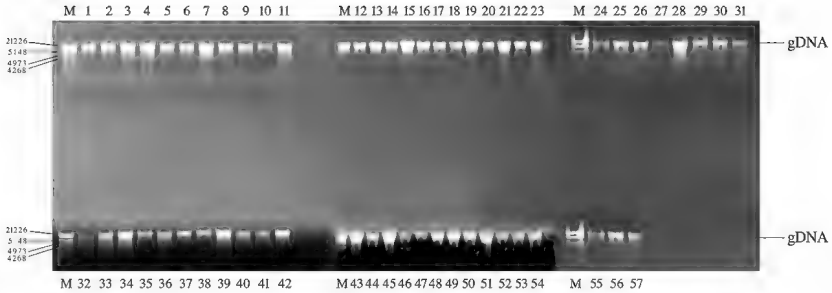

FIGURE 1. Agarose gel analysis of PCR products from genomic DNA of fungal cultures. Lane M, DNA molecular size marker (Lambda/HindIII); Lanes 1–7, 9–24, 26–28, 30, 32–35, 37–41, 43–57, *A. flavus*; Lanes 8; 25; 31; 36; 42, *A. parasiticus*; Lane 29, *A. tamarii*.

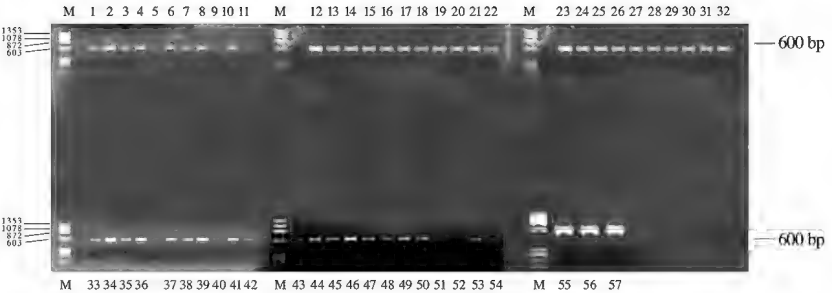

FIGURE 2. Agarose gel analysis of PCR amplification products from strains using ITS1-ITS4 primers. Lane M, PhiX174 DNA/BsuRI HAEIII marker; Lanes 1–7, 9–24, 26–28, 30, 32–35, 37–41, 43–57, *A. flavus*; Lanes 8, 25, 31, 36, 42, *A. parasiticus*; Lane 29, *A. tamarii*.

regions from the samples generated PCR products around 600 bp long. FIGURES 1 and 2 show the results of gel electrophoresis for genomic DNA isolation and PCR amplification, respectively.

A matrix comparing the ITS1 and ITS4 sequence similarities among the four *Aspergillus* reference species is depicted in TABLE 1.

Since the strains were previously identified as *Aspergillus* sect. *Flavi* using morphological and phenotypical criteria (Heperkan & Karbancioglu-Güler 2009), only *Aspergillus* sect. *Flavi* species were used for reference. Similarities between the isolate ITS sequences and the reference strain sequence of the same *Aspergillus* species are shown in TABLE 2.

We followed protocols set forth by González-Salgado et al. (2008) for differentiating *A. flavus* and *A. parasiticus* species in wheat flour. Although several PCR-based methods use aflatoxin biosynthetic genes to detect

TABLE 1. Matrix of ITS region similarities (%) for *Aspergillus* reference species

Aspergillus spp. (isolate codes) / base pairs (bp)	*A. flavus*	*A. oryzae*	*A. parasiticus*	*A. sojae*	*A. tamarii*	*A. pseudotamarii*
A. flavus (ATCC 16883) / 595						
A. oryzae (ATCC 4814) / 555	95					
A. parasiticus (NRRL 2999) / 576	98	96				
A. sojae (IFO 4386) / 576	98	96	99			
A. tamarii (JCM 2259) / 579	97	95	98	98		
A. pseudotamarii (NRRL 25517) / 598	97	91	95	95	95	

TABLE 2. Molecular identification of *Aspergillus* sect. *Flavi* isolates

No.	ITS1 / ITS4 sequence similarities (out of 5)	Similarity (%)	Isolate name	No.	ITS1 / ITS4 sequence similarities (out of 5)	Similarity (%)	Isolate name
1	3 / 3	99	*A. flavus*	30	2 / 2	98	*A. flavus*
2	3 / 3	99	*A. flavus*	31	2 / 1	96	*A. parasiticus*
3	3 / 3	99	*A. flavus*	32	2 / 1	95	*A. flavus*
4	3 / 3	99	*A. flavus*	33	3 / 2	99	*A. flavus*
5	3 / 3	99	*A. flavus*	34	2 / 2	97	*A. flavus*
6	3 / 3	99	*A. flavus*	35	1 / 4	95	*A. flavus*
7	3 / 3	99	*A. flavus*	36	3 / 3	99	*A. parasiticus*
8	3 / 3	98	*A. parasiticus*	37	2 / 3	95	*A. flavus*
9	3 / 3	99	*A. flavus*	38	4 / 2	99	*A. flavus*
10	3 / 3	99	*A. flavus*	39	3 / 5	99	*A. flavus*
11	3 / 3	99	*A. flavus*	40	2 / 2	97	*A. flavus*
12	3 / 3	99	*A. flavus*	41	2 / 3	97	*A. flavus*
13	3 / 3	98	*A. flavus*	42	1 / 3	98	*A. parasiticus*
14	3 / 3	98	*A. flavus*	43	1 / 2	99	*A. flavus*
15	2 / 2	95	*A. flavus*	44	2 / 2	97	*A. flavus*
16	2 / 2	98	*A. flavus*	45	2 / 3	99	*A. flavus*
17	2 / 2	95	*A. flavus*	46	2 / 3	98	*A. flavus*
18	2 / 1	96	*A. flavus*	47	3 / 2	99	*A. flavus*
19	2 / 2	95	*A. flavus*	48	3 / 2	99	*A. flavus*
20	3 / 1	98	*A. flavus*	49	2 / 2	95	*A. flavus*
21	2 / 3	98	*A. flavus*	50	3 / 3	99	*A. flavus*
22	3 / 2	99	*A. flavus*	51	3 / 3	98	*A. flavus*
23	2 / 2	97	*A. flavus*	52	2 / 2	99	*A. flavus*
24	3 / 5	99	*A. flavus*	53	3 / 2	99	*A. flavus*
25	3 / 1	95	*A. parasiticus*	54	2 / 1	97	*A. flavus*
26	3 / 3	99	*A. flavus*	55	3 / 4	99	*A. flavus*
27	3 / 2	99	*A. flavus*	56	2 / 1	98	*A. flavus*
28	1 / 3	95	*A. flavus*	57	2 / 2	98	*A. flavus*
29	3 / 3	99	*A. tamarii*				

aflatoxigenic fungi, it has been always a major problem to separate *A. flavus* from *A. parasiticus,* as the latter is always more easily amplified. It is believed that this might explain the high similarity in the specific primers used to amplify aflatoxin biosynthesis genes. However, ITS amplification allows us to differentiate these closely related species with greater sensitivity than single copy genes. The ITS region, which has been used for phylogenetic analysis for almost 20 years, offers distinct advantages because it customarily displays sequence variation between species as well as the minor variations within the strains of the same species. One disadvantage, however, is that the ITS region cannot differentiate toxigenic from non-toxigenic species. Potential toxigenicity of the fungi is better assessed by either monitoring several genes involved in the toxin biosynthetic pathway or analyzing mycotoxins using HPLC (Urano et al. 1992, Stroka & Anklam 2000), LC-MS (Rundberget & Wilkins 2002), and/or LC-MS/MS (Spanjer et al. 2008).

González-Salgado et al. (2008) were the first to attempt to detect *A. flavus* and *A. parasiticus* using a multicopy region (ITS). We used specific primers to amplify ITS1 and ITS2 conserved 18S rDNA regions and compared the results with the reference cultures. González-Salgado et al. (2008) initially had difficulties in differentiating *A. flavus* from *A. oryzae* and *A. parasiticus* from *A. sojae* due to the small (1–2 bp) difference separating these species. To eliminate this problem, we duplicated the amplification of some isolates. The molecular identifications were tested with 57 different fungal-isolates shown in Table 2. Results compared with the reference cultures showed a 95–99% similarity.

Henry et al. (2000), Geiser et al. (2007), Sanchez-Hervas et al. (2008), and Karthikeyan et al. (2009) have all noted the ability of molecular techniques to differentiate *Aspergillus* species. For example, *Aspergillus* sect. *Flavi* and *Nigri* species isolated from cocoa beans were first identified macro- and microscopically and the identity of the different isolates was confirmed through 5.8S-ITS rDNA sequencing after PCR amplification using the universal primers ITS4 and ITS5. *Aspergillus* spp. (including *A. flavus* and *A. tamarii*) and *Penicillium* spp. thought to represent different species were isolated and identified by ITS sequencing (Sanchez-Hervas et al. 2008).

Genetic variability among the *A. flavus* isolates was investigated by PCR amplification (using ITS1 and ITS4 primers) and sequencing of the ITS1, ITS2, and 5.8S rRNA regions from *A. flavus* isolates, yielding products of approximately 580 base pairs in length. Nucleotide sequences were compared with *A. flavus* ITS sequences in the Genbank database. Analysis of the genetic coefficient matrix derived from the ITS nucleotide sequences from *A. flavus* isolates showed that % similarities among the tested *A. flavus* strains ranged from a minimum of 12% to a maximum of 99% (Karthikeyan et al. 2009).

This molecular approach is also used to identify *Aspergillus* species from human exudates in the health sector. For example, *A. flavus, A. fumigatus,* and *A. terreus* — the most frequent causes of invasive mould infections in immunocompromised patients — have been identified by amplification of ITS1 and ITS2 conserved regions of 18S and 28S rDNA (Henry et al. 2000). Clinical application of this approach allowed the earlier diagnosis and selection of effective antifungal agents for patients with invasive aspergillosis. Most recently, serial specimens from 26 patients with documented fungal infections have been investigated by using the PCR-based ITS2 region amplification. The fungal pathogens identified were different *Aspergillus* and *Candida* species, *Rhizopus oryzae* and *Fusarium oxysporum* (Landlinger et al. 2009).

Molecular techniques have also been widely applied in an attempt to differentiate aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* species. Morphological, chemical, and molecular characterizations of 31 *Aspergillus* sect. *Flavi* isolates from Portuguese almonds were used to identify aflatoxigenic and non-aflatoxigenic strains. Morphological characterization classified 18 isolates as *A. parasiticus* and 13 isolates as *A. flavus.* Aflatoxins and cyclopiazonic acid (CPA) production of isolates investigated for the chemical characterization revealed that all *A. parasiticus* isolates were strong AFB and AFG producers but did not produce CPA. Furthermore, 8% of *A. flavus* isolates were found to produce all 3 mycotoxin groups (i.e., AFB_1, AFB_2, CPA), while 77% of *A. flavus* isolates were classified as atoxigenic. Two genes of the aflatoxin biosynthetic pathway [AFLD (NOR1), AFLQ (ORD1=ORDA)] were investigated using molecular techniques. Although the AFLD gene did not show any correlation with aflatoxigenicity, AFLQ did show some correlation. AFLD and AFLQ are not the only genes to distinguish the toxigenicity of *Aspergillus* sect. *Flavi* species. Three other genes — VER-1, OMT-1, and APA-2 coding for key enzymes and a regulatory factor in aflatoxin biosynthesis — have been used to detect *A. flavus* and *A. parasiticus* species in grains and foods. Good results have been obtained only with the DNA of the aflatoxigenic moulds, namely *A. parasiticus* and *A. flavus* (Shapira et al. 1996).

Different food and feed commodities from Mysore City in India have been examined to isolate aflatoxin-producing fungi and assess aflatoxins in commodities by targeting the AFLR and OMT aflatoxin biosynthesis genes. Although useful for detecting the *A. flavus* group, however, this protocol did not distinguish aflatoxin producers from non-producers (Somashekar et al. 2004). Another Indian study used PCR-based amplification of the aflatoxin regulatory gene AFLR for rapid detection of aflatoxigenic fungi in groundnuts and maize (Manonmani et al. 2005).

Our molecular analyses have produced results comparable to those obtained by the identification of the same strains using morphological and

phenotypical characters by Heperkan & Karbancıoglu-Güler (2009). We did detect a difference for one isolate — the aflatoxin B_1 and B_2 producer strain originally identified as *A. flavus* (isolate 36) was in fact shown by sequence analyses to represent *A. parasiticus* (Oktay & Heperkan unpublished data). The mycotoxigenic character of isolate 36 was re-analyzed with HPLC, which showed that it produced aflatoxin B_1 and B_2 but not CPA, so describing isolate 36 as an aflatoxin B_1 and B_2 producer would be more appropriate. Our result does not agree with related literature and serves as a first indication of an unusual mycotoxin pattern in *A. parasiticus*.

In conclusion, the contiguous ITS region is a very useful approach for verification of morphologically and phenotypically pre-identified *Aspergillus* sect. *Flavi* species.

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A new species and a new record of *Pyxine* (*Physciaceae*) with norstictic acid from São Paulo State, Brazil

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ABSTRACT In the course of a study of *Pyxine* species in São Paulo State, Brazil, *Pyxine jolyana* was recognized as a new species. It has norstictic acid in the medulla, a rare feature in South American species. Also, another species with norstictic acid, *P. fallax*, is recorded for the first time from the Neotropics. A world key to the *Pyxine* species that produce norstictic acid is presented.

KEY WORDS *Pyxine albovirens, P. patellaris, P. retirugella* var. *capitata, P. retirugella*, lichenized fungi

Introduction

The genus *Pyxine* differs from other foliose genera in *Physciaceae* by the epithecium becoming purple with a solution of potassium hydroxide. It shares a dark pigmented hypothecium and ascospores of the *Dirinaria*-type with *Dirinaria*, but differs from that genus by the presence of rhizines.

Other important features of the genus are the occurrence of apothecia with carbonized margins in most species, with a more or less developed and pigmented internal stipe, the presence of lichexanthone in the upper cortex in about half of the species and also the frequent pigmentation of the medulla, generally in the upper layer. These and others characters have been extensively discussed by Malme (1897), Imshaug (1957), Swinscow & Krog (1975) and Kalb (1987). About 60 *Pyxine* species are known worldwide (Kalb 2002); more than 30 species have been reported from Brazil (Jungbluth 2010).

Norstictic acid is present in the medulla of many species. Nevertheless, the only one with this acid reported for Brazil is *P. retirugella* Nyl. This species was

first cited for Brazil by Vainio (1890) followed by Zahlbruckner (1909). Kalb (1987) had the opportunity to study the material cited by Zahlbruckner (1909) and discovered it to be *P. pungens* Zahlbr. The specimens cited by Vainio (1890) could not be studied and nobody else has mentioned *P. retirugella* for South America since Zahlbruckner's work.

During our studies on the lichenized mycobiota of São Paulo State, a new species with norstictic acid in the medulla was found, which is described below. Furthermore, *P. fallax*, another species with norstictic acid, was discovered and is here reported for the first time for South America.

Material & methods

The specimens studied are from São Paulo State, Southeastern Brazil, and were studied by means of classical lichenological methods. Type specimens were kindly lent by the curators of H, S and TUR. The nomenclature of the apothecia follows Kalb (1987). The chemistry of the thalli was determined by traditional color reactions (spot tests) and thin layer chromatography (TLC) in solvent A and C, following Culberson (1972, 1974), Huneck & Yoshimura (1996) and Bungartz (2001).

Taxonomy

Pyxine jolyana Jungbluth, Kalb & Marcelli, **sp. nov.** PLATE 1

MycoBank 519465

Sicut Pyxine albovirens *sed terpenis aliis et acidum norsticticum continenti differt.*

HOLOTYPE: **BRAZIL. SÃO PAULO STATE**, Municipality of Peruíbe, Reserva Ecológica Juréia-Itatins, Núcleo Guarauzinho, 24°44′58″S, 47°02′57″W, 5 m alt., on rock next to the entrance of the reserve, 26-07-1993, leg. M.P. Marcelli 23690 (SP; isotype in Herb. Kalb).

ETYMOLOGY: The epithet *jolyana* honours Dr. Carlos Alfredo Joly, the main mentor of the BIOTA/FAPESP Program of inventory and characterization of the biodiversity of the São Paulo State.

THALLUS orbicular, saxicolous or corticolous, brownish grey, laciniate, 7 cm diam., closely adnate, 100–150 µm thick. PROXIMAL UPPER SURFACE continuous, rarely with cracks, smooth to slightly irregular and sometimes with concavities, dull, plane. DISTAL UPPER SURFACE continuous, smooth, slightly shiny, plane to concave, without a darker zone near the tips. UPPER CORTEX 10–20 µm thick. LACINIAE sublinear, irregularly to rarely dichotomously branched, contiguous to overlapping laterally, 0.5–1.0 mm wide; apices subtruncate to rounded, concave, slightly ascendant to adnate; lateral margin smooth to sinuous; axils acute. PRUINA very rare, farinaceous or forming small patches, subapical. MACULAE mainly indistinct, sparse, irregularly linear, when marginal more distinct. POLYSIDIANGIA and ISIDIA absent. SORALIA frequent, pale yellow, orbicular to capitate, submarginal; soredia powdery to granular. MEDULLA cream to pale yellow to salmon above, sulphur yellow below the soralia, lower

layer white and very thin, sometimes with K+ reddish to purple pigment present, 60–80 µm thick. Algal layer continuous, 10–20 µm thick. Distal lower surface black but paler near the tips, slightly shiny, smooth to rarely irregular. Proximal lower surface black, slightly shiny, smooth to slightly irregular. Lower cortex 15–20 µm thick. Rhizines of the same colour as the lower cortex, the apices sometimes becoming paler, mainly simple, numerous, evenly distributed, to 0.7 mm long. Apothecia absent. Pycnidia rare, laminal. Conidia sublageniform, 3.7–4.0 × ca. 1.0 µm.

Color tests: upper cortex K–, UV+ yellow; pigmented distal upper medulla K– or K+ yellow turning red, C–, KC–, P+ yellowish orange, sometimes the color of the medulla just becomes stronger; pigmented proximal upper medulla K+ yellow turning red, C–, KC–, P+ strong yellow or yellowish orange, with pigment K+ purple in oldest parts; lower medulla with negative reactions.

TLC: lichexanthone (cortex), norstictic acid, unidentified triterpenes and pigments (medulla).

Distribution– São Paulo State, littoral zone, at the municipalities of Peruíbe, São Luís do Paraitinga and Ubatuba.

Paratypes **BRAZIL. São Paulo State**, Peruíbe Municipality, border of the Guaraú River, near to the mouth of the river, 24°22′24″S, 47°00′25″W, 4 m alt., on palm stipe next to the base of the Faculdade de Ciência e Tecnologia Santa Cecília, windy and direct sun, 24-07-1988, leg. M.P. Marcelli 4014 (SP); São Luís do Paraitinga Municipality, Parque Estadual da Serra do Mar, Núcleo Santa Virgínia, in the beginning of the "Trilha do Pirapitinga" footpath, 23°20′17″S, 45°08′45″W, 915 m alt., on a tree at the border of a shadowy forest, corticolous, 14-I-2007, leg. P. Jungbluth, M.J. Kitaura, L.S. Canêz & A.A. Spielmann 1637 (SP); Ubatuba Municipality, Parque Estadual da Serra do Mar, Núcleo Picinguaba, Praia da Fazenda, 23°21′41″S, 44°50′53″W, 1 m alt., on twig of tree at the border of a Restinga forest, near the ocean, 13-I-2007, leg. P. Jungbluth, M.J. Kitaura, L.S. Canêz & A.A. Spielmann 1566B (SP).

Remarks – *Pyxine jolyana* is characterized by the presence of lichexanthone in the upper cortex, the orbicular to capitate laminal soralia, the yellow to orange pigments in the upper medulla in distal regions of the thallus and cream to salmon pigments in proximal regions. Also, some parts result in negative spot tests, others are K+ yellow turning red (norstictic acid).

It is important to note that in this species the color of the pigmented medulla is not evenly distributed throughout the thallus, and norstictic acid is not detected in TLC using just the tips of the laciniae: pieces of proximal parts of the thallus must be analyzed too, because probably higher concentrations of this acid are present in the thallus center.

Lücking et al. (2008) observed the opposite in several species of the genus *Heterodermia*: they noted that norstictic acid was produced only in the lobe tips, which might lead to misidentifications in chemical tests performed in proximal parts of the thalli.

Pyxine jolyana is the only species in the genus with lichexanthone in the upper cortex and norstictic acid in the medulla. *Pyxine fallax* and *P. retirugella* also have norstictic acid in the medulla, but both can be easily separated from the present species by the presence of atranorin in the cortex.

Pyxine albovirens (G. Mey.) Aptroot and *P. caesiopruinosa* (Tuck.) Imshaug (H!) are morphologically similar to *P. jolyana* but differ in the absence of norstictic acid.

Crystals on the upper cortex similar to those observed by Kalb (1987) in South American material of *P. caesiopruinosa* could also be observed in *P. jolyana*.

Pyxine fallax (Zahlbr.) Kalb, Bibliotheca Lichenologica 88: 315 (2004).

≡ *Parmelia fallax* Zahlbr., Ann. Mycol. 10: 381. 1912.

TYPE: Hawaiian Islands, Oahu, Kalimooa Valley, leg. Rock 89 (lectotype: W!).

Pyxine patellaris Kurok., Bull. Natl. Sci. Mus., Tokyo 12: 689. 1969.

TYPE: Bonin Islands, between Okumura and Ohgiura, Chichijima Island, along trail in the forest of *Calophyllum inophyllum Terminalia catappa*, alt. 0 100 m, leg. Hiroshi Inoue 19027 (holotype: TNS!).

Pyxine retirugella var. *capitata* Zahlbr., in Magnusson & Zahlbruckner, Ark. f. Bot. 32A(2): 59. 1945.

TYPE: United States, Hawaii, Kauai, Haena, S. of Hilo. *Pandanus* forest, 1922, leg. Skottsberg 1267 (lectotype: S!) (new synonym).

THALLUS orbicular, corticolous, grey to brownish grey, laciniate, 2.0–4.0 cm diam., adnate. PROXIMAL UPPER SURFACE continuous to cracked, slightly scrobiculate, slightly shiny, plane. DISTAL UPPER SURFACE continuous to cracked, smooth, sometimes with concavities, shiny, slightly convex to convex, with a darker zone near the tips of a few laciniae. LACINIAE sublinear, irregularly branched, contiguous to rarely laterally overlapping, 0.3–1.0 mm wide; apices rounded, sometimes subtruncate, mainly slightly concave, slightly ascending; lateral margin crenate to irregular; axils acute. PRUINA often absent or sparse, farinaceous, subapical. MACULAE distinct, abundant, irregularly linear, rarely subreticulate, marginal and laminal, starting mainly from the axils, sometimes developing into fissures. POLYSIDIANGIA and ISIDIA absent. SORALIA white, orbicular to capitate, starting as a wart which soon disintegrates apically, but continuing to expand, elevating the soralia, laminal or submarginal; soredia farinaceous. MEDULLA white. DISTAL LOWER SURFACE cream to pale brown, shiny, smooth to papillate. PROXIMAL LOWER SURFACE black, shiny, smooth. RHIZINES black, simple to irregularly branched, frequent, evenly distributed, up to 0.5 mm long. APOTHECIA of *obscurascens*-type (absent in Brazilian material), rare, 0.4–1.1 mm wide, disc epruinose, internal stipe distinct, white (Elix 2009), ascospores 13–17 × 6–8 µm (Kalb 2004, Elix 2009)]. PYCNIDIA few, laminal. CONIDIA not found [sublageniform, 3.4–3.6 × 0.5 µm (Zahlbruckner 1912, protologue)].

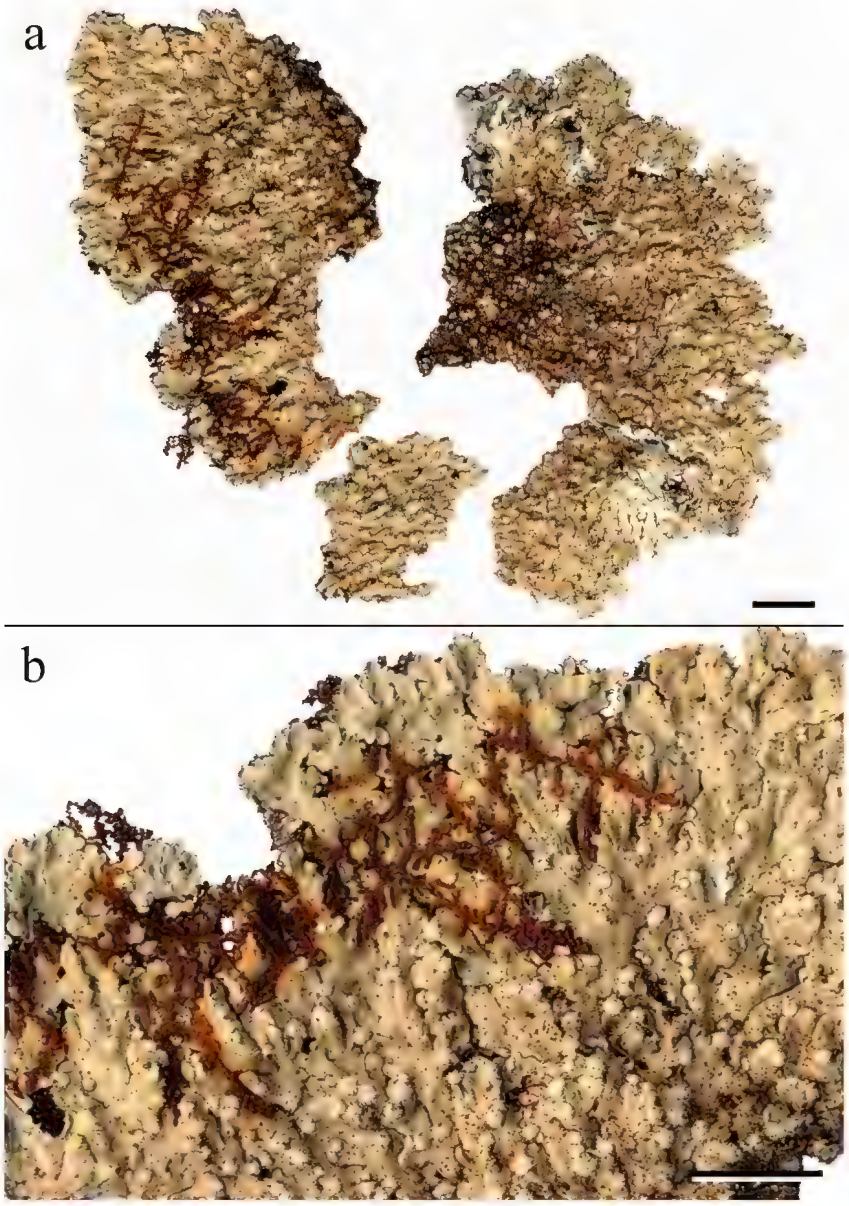

Plate 1. *Pyxine jolyana* holotype (SP).
a. Part of the holotype.
b. Part of the thallus.
Bars: 5 mm.

COLOR TESTS: upper cortex K+ yellow, UV–; medulla K+ yellow turning orange, C–, KC–, P+ orange, UV–; internal stipe K–, P–.

TLC: atranorin (cortex), norstictic acid and unidentified triterpenes (medulla).

DISTRIBUTION– Australia (Kalb 2004), Hawaiian Islands (Zahlbruckner 1912, Magnusson & Zahlbruckner 1945, as *P. retirugella* var. *capitata*), Japan (Kurokawa 1969, as *P. patellaris*), Taiwan (Kalb 2004) and South America.

SPECIMEN EXAMINED – **BRAZIL. SÃO PAULO STATE**, Praia Grande Municipality, Cidade Ocean District, 24°02′S, 46°30′W, 2 m alt., ca. 1 km after the Iemanjá's statue, 2 km after the Netuno's statue, in direction of Mongaguá Municipality, low Restinga forest, corticolous, 07-07-1988, leg. M.P. Marcelli 3303 (SP).

REMARKS– *Pyxine fallax* is characterized by atranorin in the upper cortex, orbicular, laminal to sublaminal soralia, white, K+ yellow turning orange, P+ orange (norstictic acid) medulla and *obscurascens*-type apothecia.

It is notable that in this species the soralia progressively become slightly elevated through a small base of the upper cortex (almost stalked). The same was observed in the holotype of *P. copelandii* Vain. (TUR-Vain.!), which differs from *P. fallax* by its longer ascospores (16–22 µm; Swinscow & Krog 1975), less maculate upper cortex and a different terpene array on the TLC plates.

Further similar species with white medulla and norstictic acid are *P. retirugella* (H-NYL!), which differs from *P. fallax* by the presence of polysidiangia, and *P. profallax* Kalb, which does not produce soredia (Kalb et al. 2009)

Pyxine retirugella var. *capitata* is found to be a new synonym of *P. fallax*. Rogers (1986) proposed it as a synonym of *P. retirugella*. However the lectotype of this variety (S!) has orbicular soralia instead of polysidiangia and has the same triterpene array on TLC plates as *P. fallax*. Kalb (2004) placed *P. retirugella* var. *capitata* into the synonymy of *P. asiatica* Vain.; however, *P. asiatica* does not have norstictic acid (Kashiwadani 1977, Awasthi 1982, Kalb 2004).

World key to *Pyxine* with norstictic acid present at least in some parts of the thallus or apothecia

1a. Thallus with isidia, polysidiangia or/and soredia 2
1b. Thallus without vegetative propagules 7
2a. Upper cortex K , UV + yellow, with lichexanthone ..... *P. jolyana*
2b. Upper cortex K + yellow, UV , with atranorin 3
3a. Thallus isidiate, without soredia or polysidiangia ..... *P. cylindrica* Kashiw.
3b. Thallus without isidia, with soredia or polysidiangia 4
4a. Maculae and soredia bright red [norstictic acid present only in the hypothecium] ..... *P. coccifera* (Fée) Nyl.
4b. Maculae and soredia absent or with other colors 5

5a. Thallus with polysidiangia, without soralia ..... *P. retirugella*
5b. Thallus with soralia, without polysidiangia 6
6a. Upper cortex abundantly maculate, ascospores 13–17 µm ..... *P. fallax*
6b. Upper cortex sparsely maculate, ascospores 16–22 µm ..... *P. copelandii*
7a. Medulla white throughout ..... *P. convexior* (Müll. Arg.) Swinscow & Krog
7b. Medulla at least in part pigmented 8
8a. Medulla orange to ochre throughout [norstictic acid present only in the epithecium] ..... *P. schechingeri* Kalb
8b. Medulla pale yellow or cream or orange above, white below 9
9a. Internal stipe K + purple, known only from Africa ..... *P. africana* Kalb
9b. Internal stipe K , known only from Asia ..... *P. philippina* Vain.

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Marine fungi from Sarushima Island, Japan, with a phylogenetic evaluation of the genus *Naufragella*

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Abstract Twenty-seven fungi (18 ascomycetes, 9 anamorphic fungi) were recorded from 91 driftwood samples collected from Sarushima Island, Japan. *Ceriosporopsis halima*, *Corollospora maritima*, and *Halosphaeria appendiculata* occurred most frequently in the fungal community. Other common fungi include: *Remispora maritima*, *Tirispora* sp., *Monotosporella* sp., *Phoma* sp., and *Trichocladium achrasporum*. Among the 27 fungi collected, 7 are possibly new to science. *Nemania maritima* and *Panorbis viscosus* are new records for Japan. Phylogenetic analyses of LSU rDNA sequences placed *Naufragella delmarensis* with *Nohea umiumi* in a highly supported clade. Both *Naufragella* and *Nohea* have similar morphological characters that, in combination with the molecular data, support their placement in a single genus. The new combinations *Nohea delmarensis* and *N. spinibarbata* are proposed. The anamorph teleomorph connection of *Trichocladium achrasporum Halosphaeriopsis mediosetigera* was supported with molecular data for the first time.

Key words ecology, *Halosphaeriales*, sub-tropics, aquatic fungi

Introduction

Our knowledge of lignicolous marine fungi from coastal areas of Japan is fragmentary, with only a few studies carried out with wood blocks baits in oceanic water (Tubaki 1966, 1967, 1969, 1973). These four studies documented the presence of 25 lignicolous marine fungi, including a new species, *Remispora galerita* Tubaki (Tubaki 1967). Sarushima Island, the only natural island in Tokyo Bay, was a military fort until the Second World War when it was closed to civilian access. Sarushima Island is now a tourist site and the public has easy access to the island. As no marine fungi have been reported from this small island, this study was carried out to document its marine fungal diversity.

The genera *Nohea* and *Naufragella* are closely similar in their morphology. Both genera have two types of ascospore appendages: gelatinous and fibrous. The two genera differ only in the position of the ascospore appendages. *Nohea* has gelatinous appendages attached subapically and laterally and two lateral fascicles of thin hairs. *Naufragella* has apical gelatinous appendages and a subapical crown of filaments at each apex below the gelatinous appendages. Kohlmeyer and Volkmann–Kohlmeyer (1998) opined that the differences in the positions of the gelatinous appendages (lateral in *Nohea* vs. apical in *Naufragella*) and filaments (on one side in *Nohea* vs. forming a subapical crown in *Naufragella*) are distinctive characters that can be used to separate fungi at the generic level. Phylogenetic analyses herein were carried out to test whether the genera *Nohea* and *Naufragella* are congeneric. Another closely similar genus, *Remispora,* is characterized by its wing-like apical, gelatinous ascospore appendages that unfold to form long fibrous material in water.

During this study, phylogenetic analyses of LSU rDNA sequences were carried out to elucidate the relationships among the genera *Naufragella*, *Nohea*, and *Remispora* as well as among various marine species.

Materials and methods

Collection techniques

Samples of driftwood collected from the intertidal zone of Sarushima Island, Japan, were placed in clean plastic bags and returned to the laboratory. They were examined with a dissecting microscope for marine fungi immediately upon return to the laboratory, placed in sterile humid plastic boxes for incubation, and examined periodically over 4 months of incubation. Methods used for the preparation of materials for light microscopy followed those of Jones & Hyde (1988). For each taxon, percent occurrence was calculated as: (no. of collections) × 100/(no. of samples).

Digital micrographs were obtained with a Nikon Eclipse E800 differential interference contrast light microscope and "studio viewfinder version 3" (Nikon Corporation, Japan) digital imaging system. Wood samples bearing fungi were dried at 60 °C for 24 h. To obtain single-ascospore cultures of the recorded fungi, ascomata were cut open with a sterile razor blade, and the centrum tissue containing ascospores was removed with sterile forceps and placed in sterile seawater. Small drops of this ascospore suspension were placed on GYA (10 g glucose, 1 g yeast extract, 18 g agar in 1 l seawater) in Petri dishes and incubated at 25°C in the dark. Germinated ascospores were transferred to new GYA Petri dishes with sterile forceps and incubated at 25°C in the dark. Dried fungi and isolated fungal cultures were deposited at the Extremobiosphere Research Center, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Japan and National Institute of Technology and Evaluation, Biological Resource Center (NBRC), Japan. DNA isolation, PCR reactions, cycling parameters, sequencing, sequence alignment and phylogenetic analyses were carried out as described by Abdel-Wahab et al. (2009).

DNA extraction, sequencing, and phylogenetic analysis

DNA was extracted from pure fungal mycelium using the Microbial DNA Extraction Kit (MOBIO; Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Partial LSU ribosomal DNA was amplified using primers LR0R and LR7 (Bunyard et al. 1994). PCR reactions, cycling parameters and sequencing were carried out as described by Abdel-Wahab et al. (2009). Sequences were assembled using Sequencher 4.2.2 (Gene Codes Corporation). Sequences were aligned with others retrieved from GenBank using ClustalX (Thompson et al. 1997) and optimized manually. The positions where one or more species contained a length mutation and ambiguously aligned regions were not included in the subsequent phylogenetic analysis. Nucleotide sequence phylogenies were constructed using PAUP* 4.0b10 (Swofford 2002). Maximum likelihood (ML) analyses (Felsenstein 1981) were performed using heuristic searches with the random stepwise addition of 100 replicates and tree bisection reconnection (TBR) rearrangements. The optimal model of nucleotide substitution for the ML analyses was determined using hierarchical likelihood ratio tests as implemented in Modeltest 3.7 (Posada & Crandall 1998). The model selected as the best fit for LSU rDNA data set was TrN+I+G. For the bootstrap analyses (Felsenstein 1985), 100 replicates were generated with 5 random additions and TBR. Maximum parsimony (MP) trees were obtained by 100 random addition heuristic search replicates using PAUP, and 1000 bootstrap replicates were performed employing 5 random addition heuristic searches. Posteriori probability values were obtained by using the MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) with the GTR+I+G model that was determined using MrModeltest 2.2 (Nylander 2004). Nodal supports in the Bayesian analyses were examined by posterior probabilities from five million generations that were run in four chains with sampling every 100 generations, yielding 50 000 trees, of which the first 12 500 were discarded as "burn in." Five sequences of the LSU rDNA sequences were generated in this study and were deposited at GenBank and their accession numbers are shown in Fig. 1.

Results

Twenty-seven fungi (18 ascomycetes and 9 anamorphic fungi) were identified from 122 fungal collections that were recorded from 91 driftwood samples collected from Sarushima Island, Japan. *Ceriosporopsis halima* (17.6 %), *Corollospora maritima* (43.9 %) and *Halosphaeria appendiculata* (12.1 %) occurred most frequently. Other common fungi include: *Remispora maritima* (5.5 %), *Tirispora* sp. (8.8 %), *Monotosporella* sp. (5.5 %), *Phoma* sp. (7.7 %) and *Trichocladium achrasporum* (5.5 %). Among the 27 fungi recorded during the present study, 7 fungi (representing 25.9 % of the recorded fungi) are possibly new to science. *Nemania maritima* and *Panorbis viscosus* are reported from Japan for the first time. Representatives of the *Halosphaeriales* predominated in the fungal community, comprising 27 species (59.3 %). Hyphomycetes, represented by 8 species, comprised the major anamorphic group, and coelomycetes were represented by one species of *Phoma* (Table 1).

TABLE 1. Frequency of occurrence of marine fungi collected from Sarushima Island

FUNGI	%
Ascomycota	
Botryosphaeria sp.	1.1
Ceriosporopsis halima Linder	17.6
**Ceriosporopsis* sp. 1	1.1
**Ceriosporopsis* sp. 2 (MF 985)	3.3
Corollospora maritima Werderm.	43.9
**Dryosphaera* sp.	1.1
Halosphaeria appendiculata Linder	12.1
Halosphaeriopsis mediosetigera (Cribb & J.W. Cribb) T.W. Johnson	2.2
Leptosphaeria sp.	1.1
Lulworthia grandispora Meyers	1.1
Marinospora calyptrata (MF986)	3.3
**Morakotiella* sp. (MF 980)	2.2
Nemania maritima Y.M. Ju & J.D. Rogers	2.2
Panorbis viscosus (I. Schmidt) J. Campb. et al.	1.1
Nohea delmarensis (MF982)	1.1
Remispora maritima (MF988)	5.5
**Remispora* sp. (MF987)	1.1
**Tirispora* sp. (MF960)	8.8
Anamorphic fungi	
Cirrenalia macrocephala (Kohlm.) Meyers & R.T. Moore	1.1
Halazoon fuscus (I. Schmidt) Abdel-Wahab et al.	2.2
**Monotosporella* sp. (MF984)	5.5
Phoma sp.	7.7
Trichocladium achrasporum	5.5
Trichocladium melhae E.B.G. Jones et al.	1.1
Xylomyces sp.	1.1
Veronaea japonica Arzanlou et al. (MF991)	1.1
Unknown synnematous fungus	1.1

*Possibly new fungi

Total numbers: samples 91; fungal collections 122; species 27; ascomycetes 18; anamorphic fungi 9; fungi per sample 1.3.

Phylogenetic analyses of *Halosphaeriales* LSU rDNA dataset

The partial LSU rDNA sequences of *Marinospora calyptrata*, *Naufragella delmarensis*, *R. maritima*, and *T. achrasporum* were aligned with representatives of the *Halosphaeriales* along with representatives of the *Microascales* and *Xylariales*. In total, the dataset included 36 taxa of which 31 belong to *Halosphaeriales*, one to *Microascales,* and four to *Xylariales* that were used as outgroup taxa. The dataset consisted of 460 total characters, of which 49 gaps were excluded, 250 characters were constant, 48 variable characters were parsimony–uninformative, and 113 were parsimony informative characters.

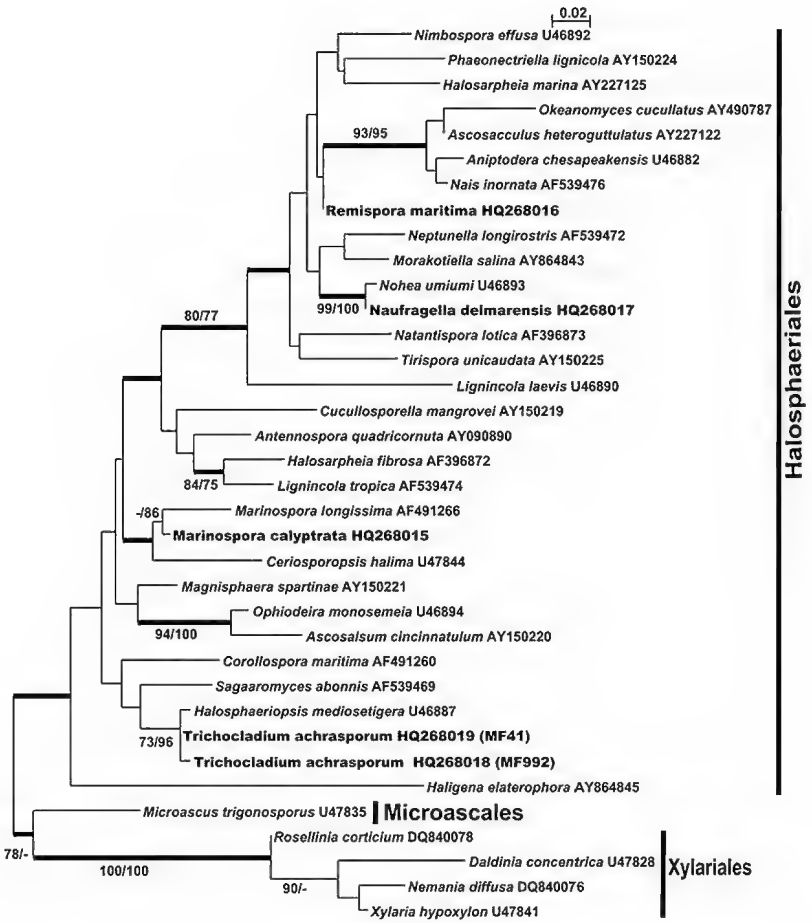


FIG. 1: Maximum likelihood phylogenetic tree (ln likelihood −3,008.01) shows relationships of *Marinospora calyptrata, Naufragella delmarensis, Remispora maritima,* and *Trichocladium achrasporum* and representatives of halosphaeriaceous ascomycetes based on LSU rDNA. The numbers indicate pp values > 95% (in bold), ML bootstrap and MP bootstrap values > 70%. Sequences generated during the present study are set in bold font.

The heuristic search generated 24 most parsimonious trees, all 520 steps long, and with a consistency index of 0.4462, a retention index of 0.6449, and a rescaled consistency index of 0.2877. Maximum likelihood analysis produced one tree with an –ln likelihood score of 3,008.01 (FIG. 1). Most parsimonious (MP) and Bayesian analyses produced trees (not shown) similar to the one in FIG. 1.

Taxonomic notes

Naufragella Kohlm. & Volkm.-Kohlm.

Kohlmeyer & Volkmann-Kohlmeyer (1998) established the genus *Naufragella* Kohlm. & Volkm.-Kohlm. to accommodate a new fungus, *N. delmarensis*, and also transferred *Remispora spinibarbata* to the new genus. During this study *N. delmarensis* was isolated, described, photographed (Figs 2–5), and sequenced.

The genera *Naufragella* and *Remispora* Linder share common morphological characters. Barghoorn & Linder (1944) established the genus *Remispora* to accommodate halosphaeriaceous fungi with exosporic ascospore appendages that consist of a fibrous component in an amorphous matrix. Fragmentation of the sheath gives rise to wing-like fibrous polar appendages (Jones & Moss 1978, Johnson et al. 1984, Jones et al. 2009). Recent phylogenetic analyses revealed that *Remispora* is polyphyletic (Jones et al. 2009).

In contrast, *Naufragella* and *Nohea* both have two types of appendages: (a) gelatinous, forming a band or veil at each apex and (b) a crown of delicate filaments below each apex (Kohlmeyer & Volkmann-Kohlmeyer 1998). Phylogenetic analyses place the type species of both genera, *Nohea umiumi* and *Naufr. delmarensis,* within a highly supported clade (99/100/100 for ML/MP/Bayesian pp respectively) that forms a sister group to *Morakotiella salina* (C.A. Farrant & E.B.G. Jones) Sakay. and *Neptunella longirostris* (Cribb & J.W. Cribb) K.L. Pang & E.B.G. Jones. I conclude that the genera, *Nohea* and *Naufragella* are congeneric and that, within the *Halosphaeriales,* ascospore appendage position is not a reliable character with which to separate fungi at the generic level, contrary to the opinion of Kohlmeyer & Volkmann-Kohlmeyer (1998). *Naufragella spinibarbata* is very similar to *Naufr. delmarensis,* and the two species differ only in ascospore dimensions and appendage shape and length. *Naufragella spinibarbata* was not available for sequencing and further study is needed to confirm its phylogenetic relationships but for the time being it should returned to *Nohea.*

Based on the morphology and the molecular results presented here, the following two combinations are proposed:

Nohea spinibarbata (Jørg. Koch) Abdel-Wahab, **comb. nov.**

MycoBank MB 519505

≡*Remispora spinibarbata* Jørg. Koch, Nordic Journal of Botany 8: 517, 1989.

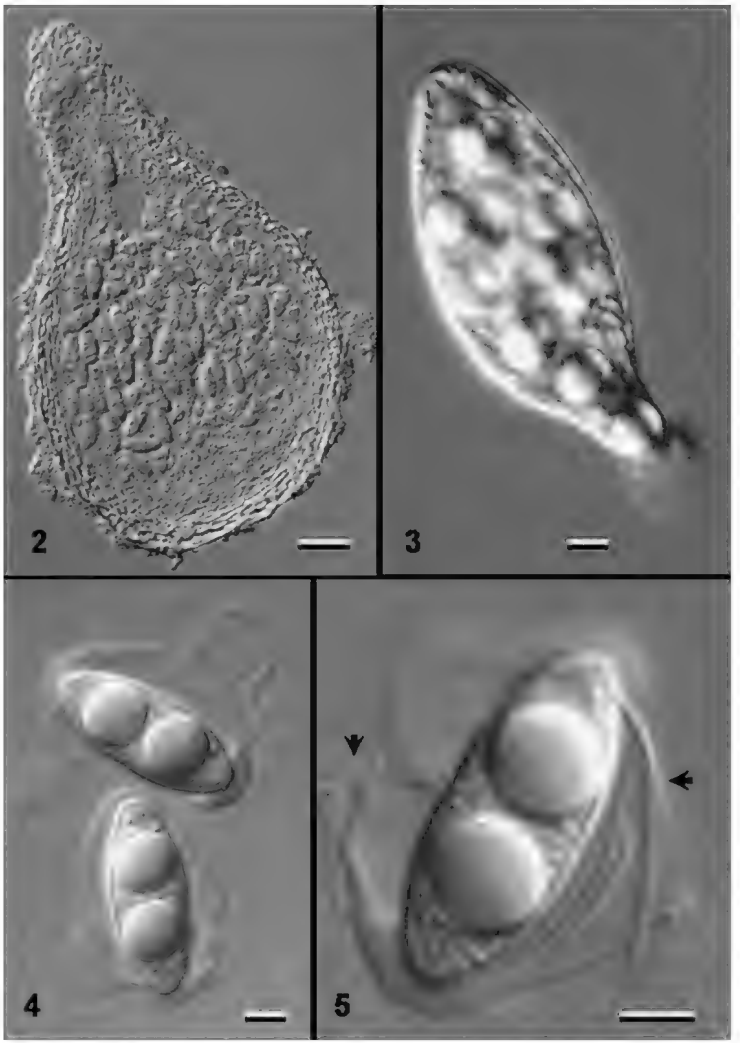
≡*Naufragella spinibarbata* (Jørg. Koch) Kohlm. & Volkm.-Kohlm. Systema Ascomycetum 16: 11, 1998.

Nohea delmarensis (Kohlm. & Volkm.-Kohlm.) Abdel-Wahab, **comb. nov.**

MycoBank MB 519504 Figs 2–5

≡*Naufragella delmarensis* Kohlm. & Volkm.-Kohlm., Systema Ascomycetum 16: 10, 1998.

Ascomata 230–260 µm high, 250–270 µm in diam, erumpent to superficial, ostiolate with short neck, periphysate, coriaceous, hyaline to cream colored,



Figs 2–5: *Nohea delmarensis*. Differential interference contrast light micrographs. 2. Vertical section through ascoma. 3. Immature ascus. 4–5. Ascospores with elaborate two types of appendages (Fig. 5, arrowed). Bars: 2 – 50 µm, 3–5 – 5 µm.

solitary or gregarious (Fig. 2). Peridium 12–15 µm thick consisting of 4–6 layers of flattened polygonal cells. Neck 50–90 µm high, 40–48 µm in diameter. Asci 58–70 × 18–27 µm, 8-spored, thin-walled, clavate, pedunculate, deliquescing at maturity (Fig. 3). Ascospores 19–21 × 8.5–9 µm, one-septate, not constricted at the septum, ellipsoidal, with tapered ends. Appendages of two types: (1) polar,

gelatinous, forming a cap-like structure at the apex and lying flat against the lateral wall forming two unequal wings, wings unfurling in water to form long sticky filaments and (2) sub-polar crown of spines 15–20 µm long (Figs 4–5).

Marinospora calyptrata (Kohlm.) A.R. Caval. Figs 6–10

During the course of this study, the type species of *Marinospora*, *M. calyptrata*, was recorded, described, photographed, isolated and sequenced.

Ascomata 650–850 µm high and 900–1150 µm wide, deeply immersed with long neck growing through the substrate to the surface, subglobose to fusiform or flattened globose. Asci 120–180 × 24–41 µm, 8-spored, clavate with beaked apex, deliquescing at maturity (Fig. 6). Catenophyses present. Ascospores 26–35 × 10–16 µm, one-septate, slightly constricted at the septum, ellipsoidal, hyaline, rough walled (only visible at 1000×). (Figs 7, 9, 10), enclosed in a sheath, provided with four to five obclavate, tapering appendages. Appendages straight or curved, one at each ascospore apex and two to three around the mid-septum, 14–26 µm long, 5–8 µm wide at base; annulated along appendage length and bearing a terminal inverted cap (Figs 7–10).

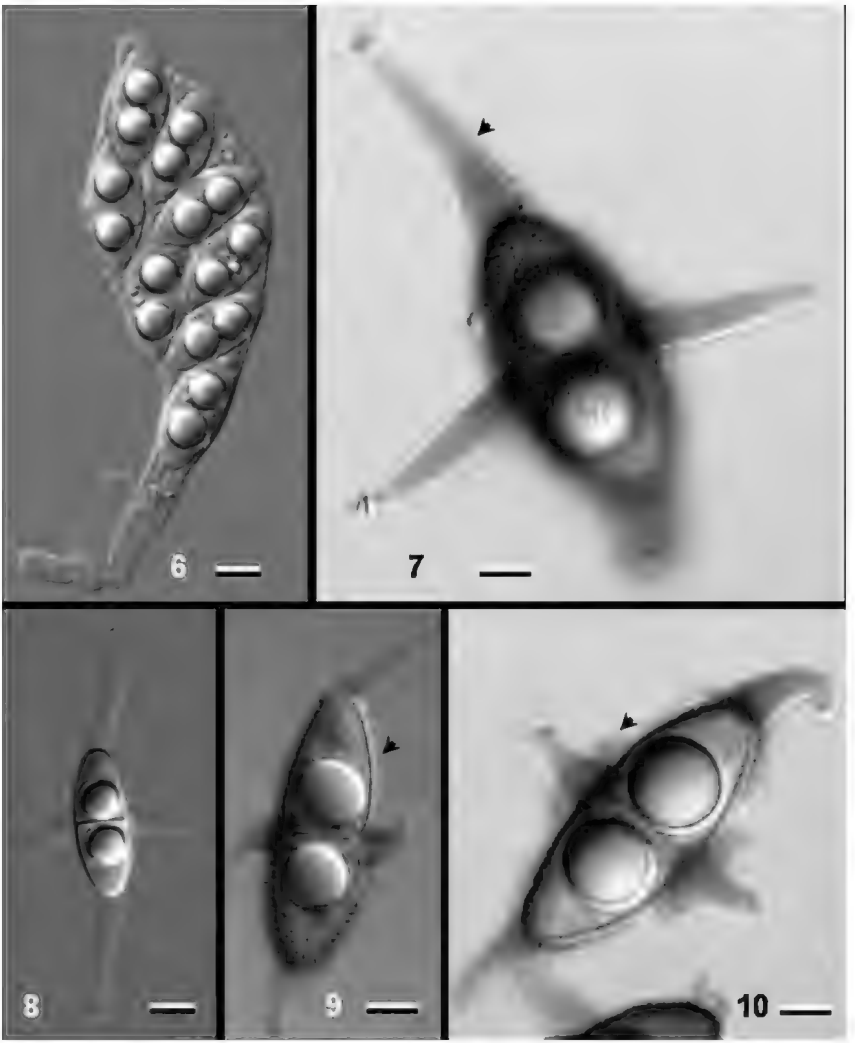
Note: *Marinospora longissima* was considered conspecific with *M. calyptrata* (Kohlmeyer & Kohlmeyer 1979). However, Jones & Moss (1980) separated the two species based on the presence of a mucilaginous envelope in *M. longissima* and consistently longer apical appendages. Phylogenetic analyses of LSU rDNA sequences of *M. calyptrata* and *M. longissima* confirm that they are distinct species and this is in agreement with previous study carried by Sakayaroj et al. (2004).

New morphological features of *M. calyptrata* observed during this study include: (1) presence of a roughened ascospore wall (Fig. 9, arrowed) surrounded by a mucilaginous layer (Fig. 10, arrowed); (2) ascospore appendages are annulated along their length (Fig. 7, arrowed). *Marinospora calyptrata* grouped consistently with *M. longissima* and *Ceriosporopsis halima*, based on molecular data (Fig. 1).

Remispora maritima Linder Figs 11–16

During the course of this study, the type species of *Remispora*, *R. maritima*, was isolated, photographed, and sequenced.

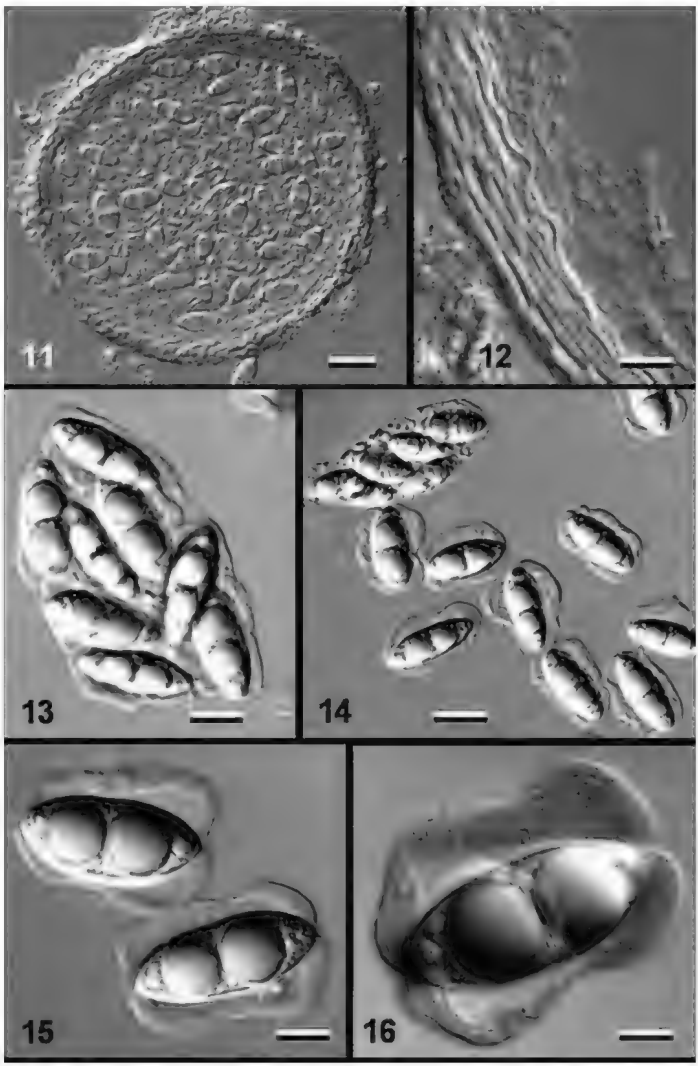
Ascomata 180–220 µm high, 250–310 µm in diam, erumpent to superficial, papillate, ostiolate, coriaceous, hyaline to cream colored (Fig. 11). Peridium 18–24 µm thick, consisting of 5–7 layers of flattened polygonal cells (Fig. 12). Neck 45–70 µm high, 60–68 µm in diam. Asci 90–120 × 26–48 µm, 8-spored, thin-walled, broadly clavate, pedunculate, deliquescing at maturity (Fig. 13). Ascospores 25–28 × 10–12 µm, one-septate, not constricted at the septum, ellipsoidal, hyaline, appendaged; at first surrounded by a gelatinous sheath



Figs 6–10: *Marinospora calyptrata*. 6. Ascus. 7–10. Ascospores with annulations along the appendages (Fig. 7, arrowed), roughened wall (Fig. 9, arrowed), and gelatinous sheath (Fig. 10, arrowed). Bars: 6,8 = 10 µm, 7,9,10 = 5 µm.

that unfolds (Figs 14–15), remaining attached at both ends of the ascospore; appendages later elongate and forming numerous fiber-like elements embedded in the gelatinous material (Fig. 16).

Note: Phylogenetic analyses of ribosomal gene sequences indicate that *Remispora* is a polyphyletic genus (Jones et al. 2009). *Remispora maritima* seems



FIGS 11–16: *Remispora maritima*. 11. Section through the ascoma. 12. Section through part of the ascomata showing peridium structure. 13. Ascus. 14–16. Ascospores. Bars: 11 = 25 µm, 12 = 5 µm, 13–14 = 10 µm, 15–16 = 5 µm.

to be cosmopolitan in its distribution but occurs more frequently in temperate regions (Kohlmeyer & Kohlmeyer 1979). In the LSU analyses in this paper, *Remispora maritima* was basal to a node containing *Aniptodera chesapeakensis*, *Ascosacculus heteroguttulatus, Nais inornata,* and *Okeanomyces cucullatus* (FIG. 1).

Trichocladium achrasporum (Meyers & Moore) Dixon ex Shearer & J.L. Crane

Shearer & Crane (1977) discovered the connection between *Halosphaeriopsis mediosetigera* and *T. achrasporum* through single spore cultural connection. That anamorph–teleomorph connection is supported at molecular level for the first time herein. Phylogenetic analyses of LSU rDNA sequences of two isolates of *T. achrasporum* that were isolated from Japan (MF992) and Egypt (MF41) were consistently grouped with the teleomorph *H. mediosetigera* with high bootstrap support (73/96/86 for ML/MP/Bayesian pp respectively). Apparently there are several varieties of *T. achrasporum*. The type specimen has conidial dimensions 17–21(–32) × (8–)10–13 (–16) µm (Meyers & Moore 1960), while Kohlmeyer & Volkmann-Kohlmeyer (1991) reported a wider range of conidial dimensions (–15)20–34(–45) × (8–)10–24 µm. The isolates *T. achrasporum* from Egypt and Japan have similar conidial dimensions (14–32 × 8–18 µm) that agree with those of the type specimen.

Goh & Hyde (1999) monographed *Trichocladium*, accepting 18 species and describing a number of new species. They referred another 22 *Trichocladium* names to other genera, e.g. *Bactrodesmium, Hemispora,* and *Pithomyces*. Several *Trichocladium* species have been described from aquatic environments (Crane & Shearer 1978, Kohlmeyer & Volkmann-Kohlmeyer 1995, Hyde & Goh 1998, 1999, Goh & Hyde 1999, Hyde et al. 1999, Jones et al. 2001). *Trichocladium achrasporum* differs from the lectotype species, *T. asperum* Harz, by having sporodochioid conidiomata (Meyers & Moore 1960). Kohlmeyer & Kohlmeyer (1979), however, considered that the degree of conidiophore aggregation in *T. achrasporum* could not be considered as a sporodochium and so accepted its relocation in *Trichocladium* from the genus *Culcitalna* Meyers & R.T. Moore. Currently, there are seven marine *Trichocladium* species (Jones et al. 2009).

Discussion

Ceriosporopsis halima and *Corollospora maritima* were the most frequently collected fungi during this study and these results are similar to those of previous studies that have been carried out in Japan (Tubaki 1966, Nakagiri et al. 1999). Both fungi are cosmopolitan species that have been recorded from various continents (Cribb & Cribb 1956, Kohlmeyer & Kohlmeyer 1979, Miller & Whitney 1981, Lintott & Lintott 1982, Zanial & Jones 1984, Jones 1985, Shearer & Burgos 1987, Hyde & Jones 1989, Tan & Leong 1992, Abdel-Aziz 2010).

Halosphaeria appendiculata was frequently recorded during the present study. This species was commonly recorded from San Juan Island (Jones 1985) and from sea foam samples in Japan (Nakagiri 1989). Although cosmopolitan in its distribution, *H. appendiculata* tends to occur more frequently in temperate water (Kohlmeyer & Kohlmeyer 1979).

Acknowledgements

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Paraconiothyrium babiogorense sp. nov., a new endophyte from fir club moss *Huperzia selago* (*Huperziaceae*)

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Abstract — *Paraconiothyrium* is a recently established genus within the order *Pleosporales*. Species from this genus are commonly associated with plants but can also be found in soil samples and be parasitic on fungi. Several isolates of a *Paraconiothyrium* sp. were obtained from *Huperzia selago* in Poland. Strains were characterized based on morphological characteristics and molecular data (SSU rDNA, ITS1, 5.8S rDNA, ITS2). Based on its unique morphology and DNA phylogeny, isolates were described as a new species: *Paraconiothyrium babiogorense*, which represents the first report of a *Paraconiothyrium* species from fir club moss. Comparison of characters with other *Paraconiothyrium* species is provided as well as a signature sequence for the new species.

Key words *Paraphaeosphaeria, Coniothyrium*

Introduction

Huperzia selago (≡ *Lycopodium selago* L.), fir club moss, is a member of the *Huperziaceae* (Rothmaler 1944, 1962). Both gametophytes and sporophytes of this plant are colonized by endophytic fungi (Higgins et al. 2007, Winther & Friedman 2008). Recently, some *Paraconiothyrium*-like, endophytic strains were isolated from healthy-looking propagules (e.g., bulbils, gemmae) of *Huperzia selago* collected in the Babia Góra National Park, Poland.

Paraconiothyrium comprises 9 species to date (Damm et al. 2008). The genus was established by Verkley (Verkley et al. 2004) to accommodate two species of *Coniothyrium*-like anamorphs, *Paraconiothyrium minitans* (W.A. Campb.) Verkley and *P. sporulosum* (W. Gams & Domsch) Verkley. The genus also comprised four newly described species: *Paraconiothyrium estuarinum*

Verkley & M. da Silva, *P. brasiliense* Verkley, *P. cyclothyrioides* Verkley, and *P. fungicola* Verkley & Wicklow. Damm et al. (2008) later described *P. variabile* Riccioni et al. and *P. africanum* Damm et al., and transferred *Microdiplodia hawaiiensis* Crous as *P. hawaiiense* (Cross) Damm et al. as indicated by ITS and SSU sequence analyses. *Paraconiothyrium* belongs to the order *Pleosporales*, class *Dothideomycetes* (Verkley et al. 2004, Damm et al. 2008). Based on SSU and ITS phylogeny, the teleomorph state of *Paraconiothyrium* belongs probably to the genus *Paraphaeosphaeria* s. str. (Câmara et al. 2001, Verkley et al. 2004).

Paraconiothyrium is characterized by presence of eustromatic, simple or complex, rarely pycnidial conidiomata. Conidiogenous cells are discrete or integrated, phialidic or sometimes percurrent. Conidia are aseptate or one-septate, thin-walled and generally smooth-walled, hyaline when liberated, later brown (Verkley et al. 2004).

Most *Paraconiothyrium* isolates to date have been obtained from woody plants (Damm et al. 2008). However, *P. minitans* and *P. fungicola* can also be fungicolous (Campbell 1947, Whipps & Gerlagh 1992, Verkley et al. 2004,), *P. sporulosum* was found in soil samples (Damm et al. 2008), and *P. estuarinum* was detected in an estuarine sediment (Verkley et al. 2004). Recently, *P. brasiliense* and *P. sporulosum* were determined as dominant endophytes of the mistletoe *Phoradendron perrottetii* (*Viscaceae*) from Brazil (Abreu et al. 2010). Damm et al. (2008) suggested that *Paraconiothyrium* was a commonly occurring fungal genus and that it was more often isolated from warm regions like South Africa, Italy, Brazil, Papua New Guinea, Hawaii (USA), and Turkey (Verkley et al. 2004, Crous & Groenewald 2006, Göre & Bucak 2007, Riccioni et al. 2007).

The aim of the present study was to evaluate the taxonomic position of *Paraconiothyrium*-like isolates from *H. selago* propagules collected from an alpine site in Babia Góra National Park, using morphological observations and analysis of ITS and SSU rDNA sequences.

Materials & methods

Isolates

Symptomless *Huperzia selago* propagules from nine different plant individuals were collected from one site in Babia Góra National Park (49°34'24"N 19°31'46"E), Beskidy Mountains, Poland. After transfer to the laboratory, the propagules were cleaned of remnants of soil and other plant leaves, rinsed in tap water with detergent, and then surface disinfected according to the protocols of Szypuła et al. (2005). In the first step propagules were subsequently rinsed for 1 min in the following solutions: 0.01 M hydrochloric acid (HCl), 5% sodium hypochlorite (NaOCl) and 2.4 mM citric acid. In the second step 70% ethyl alcohol (C_2H_5OH) for 1 min, 5% sodium hypochlorite (NaOCl) for 15 min, and 7% hydrogen peroxide (H_2O_2) for 10 min were used.

After surface sterilization propagules were plated onto potato-dextrose agar (2% PDA, Fluka® Analytical) and synthetic medium with yeast extract (BTL, Łódź, Poland)

containing 1 g L^{-1} ammonium nitrate (NH_4NO_3), 1 g L^{-1} ammonium sulfate [$(NH_4)_2SO_4$], 4 g L^{-1} potassium phosphate dibasic (K_2HPO_4), 2 g L^{-1} potassium phosphate monobasic (KH_2PO_4), 1 g L^{-1} sodium chloride NaCl, 10 g L^{-1} glucose,

1 g L^{-1} yeast extract (Difco, USA), and 15 g L^{-1} Bacto™ Agar (Becton, Dickinson and Company, USA). The pH was adjusted to 4.55 prior to sterilization by using 10% citric acid. The sterilization was carried out in an autoclave at 121° C under increased pressure of 1 bar for 15 min. The cultures were incubated at room temperature (~17°C) for 4 weeks. Reference strains of isolated fungi are maintained in herbarium (WA17577, WA17612-WA17619) and culture collection (CBS128292).

Morphology

The strains were studied on 2% Potato Dextrose Agar (PDA, Fluka® Analytical). Petri dishes were incubated at room temperature for 2–4 weeks. To enhance sporulation, autoclaved fir club moss fragments were placed on 2% PDA medium. Growth characteristics were studied on PDA plates incubated in dark at room temperature. Sporulating structures were mounted in Lactophenol Mounting Medium (Amann's fluid; Stevens 1974) and observed using Nikon Eclipse 600 and Nikon SMZ800 microscopes. Digital images were recorded with Nikon DX 1200 and Canon EOS 450D. The structures were measured using Coolview v.1.6.0 software.

DNA extraction & sequencing

Total genomic DNA was extracted from the fresh mycelium grown on PDA plates using Genomic Mini AX PLANT Kit (DNA-Gdańsk, Gdynia, Poland). The internal transcribed spacer region (ITS; ca. 0.5 kb) and fragment of 18S rDNA (SSU rDNA; ca. 1.0 kb) were amplified by PCR. Forward primer ITS1-f and reverse primer ITS4 were used to amplify the ITS region (Gardens & Bruns 1993). Forward primers 402 (5': GCT ACC ACA TCC AAG GAA GGC), 1144 (5': GCC TGC GGC TTA ATT TGA CTC) and reverse primers 1308 (5': CTC GTT CGT TAA CGG AAT TAA), R (5': TGA TCC TTC TGC AGG TTC ACC) were used to amplify SSU rDNA. PCR protocols followed Korniłłowicz-Kowalska et al. (2006). Forward and reversed sequences were matched using BioEdit Sequence Alignment Editor v. 7.0.0 (Hall, 1999).

Phylogenetic analysis

Pairwise and global alignments of ITS region and SSU rDNA were performed in BioEdit Sequence Alignment Editor v. 7.0.0 (Hall, 1999). Phylogenetic trees were obtained from the data using maximum parsimony (MP), neighbor-joining (NJ) in PAUP* v. 4.0b10 (Swofford 2002) and Bayesian analysis (BA) in MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001). Tree robustness was evaluated by 1000 replicate bootstrap analysis. The Akaike Information Criterion (AIC) implemented in Modeltest 3.7 (Posada & Crandall 1998) was used to select the model that best fit each dataset. BLAST searches in GenBank with ITS region sequences were performed using the blastn algorithm. For phylogenetic analysis *Helminthosporium velutinum* Link (AF145704) and *Helminthosporium solani* Durieu & Mont. (AF163089) for the ITS dataset and *Peziza echinospora* P. Karst. (AF006309) for the 18S rDNA dataset were used as outgroups like in research by Damm et al. (2008). GenBank accession numbers used in these studies are indicated on the phylogenetic trees.

A species specific, signature DNA sequence in the Internal Transcribed Spacer 1 (ITS1) was determined basing on ITS region alignment. The accuracy of characteristic sequence identification was verified using the BLAST algorithm against the whole GenBank database.

Results

Morphological observations

Morphological characters are presented in TABLE 1, where they are compared with other closely related species. They are also presented in the taxonomic description.

Phylogenetic analysis

The SSU region dataset contained 36 taxa and 933 characters (including gaps) and 92 characters were parsimony informative. After heuristic search, three most parsimonious SSU trees (length: 262 steps) were retained and the strict consensus tree was calculated using bootstrap 50% majority-rule. The ITS region alignment of 52 taxa contained 522 characters (including gaps), of which 179 characters were parsimony informative. After heuristic search, four most parsimonious ITS trees (length: 423 steps) were retained, and the strict consensus tree was calculated using bootstrap 50% majority-rule. The topologies of the ITS trees using MP and BA were similar (FIGS 1 and 2). However, in NJ analysis *P. fungicola* was placed in outer position to all other *Paraconiothyrium* species, and *P. sporulosum*, *P. minitans*, and two *Paraphaeosphaeria* spp. formed a sister group separate from the to the remaining *Paraphaeosphaeria* spp. Although we did not resolve phylogenetic relationships within the *Paraphaeosphaeria/ Paraconiothyrium* clade, the main species groups were the same as in Verkley et al. (2004).

The genetic variability of ITS sequences between different strains of *P. babiogorense* was very low (less than 1%). These ITS sequences revealed the highest similarity to *Coniothyrium fuckelii* Sacc. (AY904055), *Paraconiothyrium* sp. (EU709779), *Coniothyrium wernsdorffiae* Laubert (AY904058), and *Coniothyrium* sp. (GU062312; e-value = 0.0; maximum identity = 95% all). The SSU sequence of our strain revealed the highest similarity to *Bipolaris sorokiniana* (Sacc.) Shoemaker (DQ337383), *Paraphaeosphaeria michotii* (Westend.) O.E. Erikss. (AF250817), *Paraphaeosphaeria* sp. (AB096264), and *Paraconiothyrium* sp. (AB303550; e-value = 0.0; maximum identity = 99% all).

The phylogenetic analysis of the SSU dataset confirmed that our isolates are placed in the *Paraconiothyrium/Paraphaeosphaeria* clade (FIG. 1) as defined by Verkley et al. (2004). The phylogenetic tree based on ITS dataset showed that strains from *H. selago* are placed on sister branch to two other *Paraconiothyrium* sp. from Polish club mosses (FIG. 2). However, without detailed information on morphology of these two other strains, we decided not

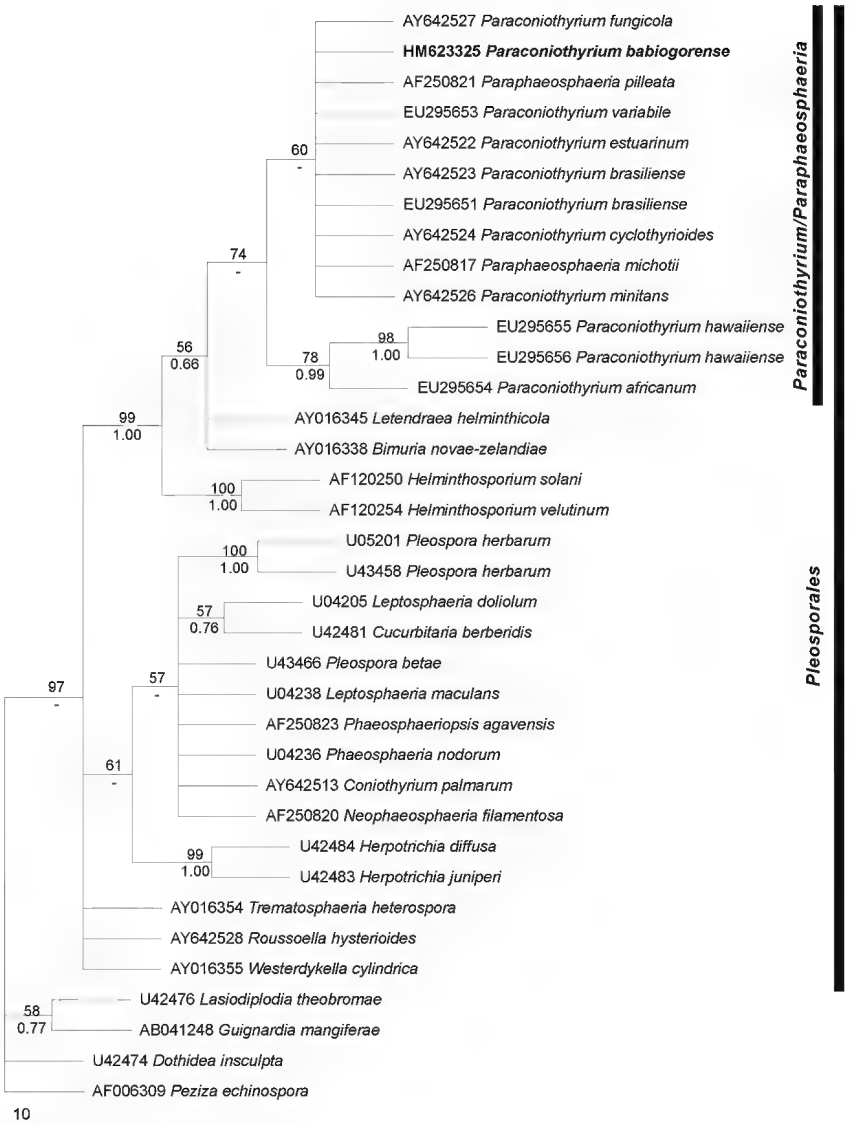

FIG. 1. Strict consensus tree obtained from partial SSU nrDNA sequences data of selected *Dothideomycetes* species. Numbers above branches indicate bootstrap support values inferred by maximum parsimony analysis (1000 replicates; length: 277 steps, CI: 0.661, RI: 0.796, RC: 0.526, HI: 0.339); numbers under branches indicate Bayesian posterior probability values. Only values higher than 50% are shown.

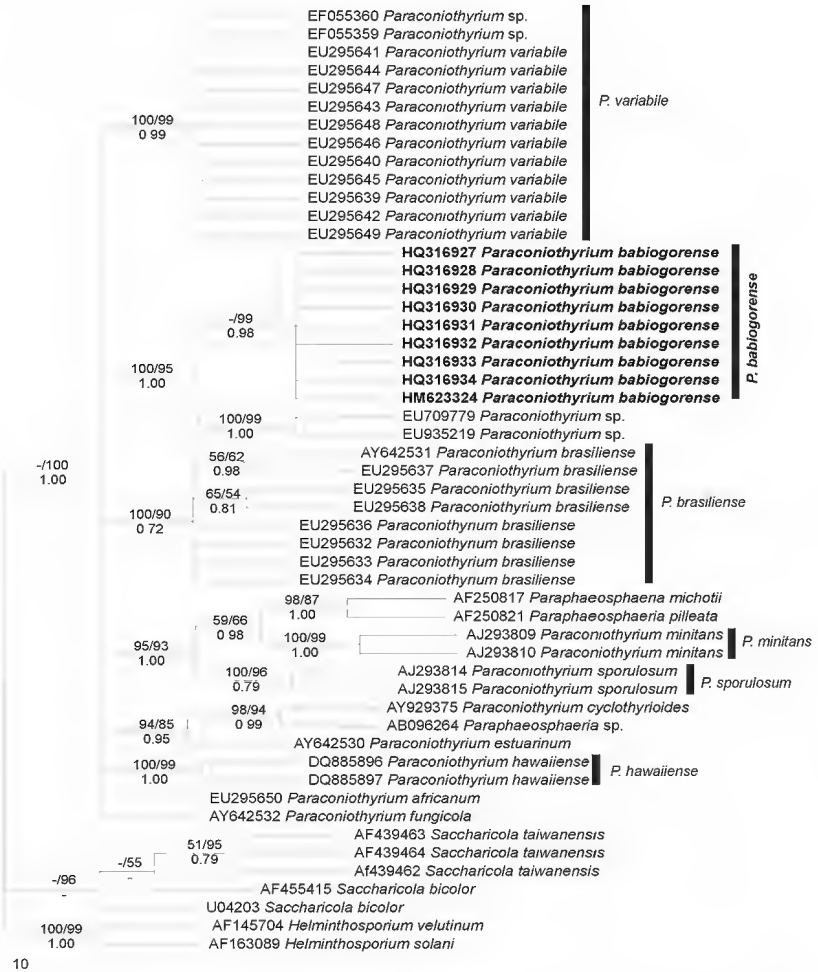

FIG. 2. Strict consensus tree obtained from ITS region sequences data of *Paraconiothyrium*/*Paraphaeosphaeria* species. Numbers above branches before slash indicate bootstrap support values inferred by neighbor-joining analysis (1000 replicates); numbers above branches after slash indicate bootstrap support values inferred by maximum parsimony analysis (1000 replicates; length: 423 steps, CI: 0.650, RI: 0.894, RC: 0.581, HI: 0.350); numbers under branches indicate Bayesian posterior probability values. Only values higher than 50% are shown.

to treat all of them as the same species. Taking into account the phylogenetic position of strains WA0000017577, WA0000017612-WA0000017619, their distinct morphological characters, and low ITS sequence similarity to other *Paraconiothyrium* representatives (less than 95%), we decided to describe them as a new, separate species, *Paraconiothyrium babiogorense*.

Taxonomy

Paraconiothyrium babiogorense Budziszewska, **sp. nov.** PLATE 1

MYCOBANK 519253

Coloniae in PDA ad temp 17°C luteae, reverso brunneae; Conidiomata eustromatica, complexa, globosa (0.2)0.3 0.5(0.9) mm diametro; Cellulae conidiogenae discretae, phialidicae, ampulliformes, hyalinae vel pallide brunneae, 5 7(9) × 3 5 µm; Conidia tempore liberationis hyalina, deinde luteo-fusca, cylindrica vel breviter cylindrica, glabra, aseptata, interdum uniseptata, guttulata, 1 2(3) × (7)8 9(10) µm.

TYPE: Poland, Babia Góra National Park, Carpathians Mountains (49°34'24"N, 19°31'46"E; WGS84 system) from healthy propagules of *H. selago*, 10 October 2009, *J. Budziszewska*; **holotype,** WA17577 (dried culture); **isotypes**, WA17612 WA17619 (dried cultures); **ex-holotype,** CBS128292 (lyophylised culture).

SIGNATURE SEQUENCE: 5' CCCCTGGGGGCGTGGGCGTCTCCCGGCGTCCTCTCTCCTG 3'

ETYMOLOGY: Named after the place of origin — the Babia Góra National Park, Poland.

Colonies on PDA reach 5 mm after 7 days and 25 mm after 28 days (~17°C, dark). Colonies are Colonial-Buff to Deep Colonial-Buff and reverse is Honey-Yellow to Isabella in color (Ridgway 1912). Conidiomata eustromatic, complex, mostly submerged in the agar, but also superficial on club moss fragments, globose to subglobose, dark brown to black, ostioles absent, (0.2–)0.3–0.5 (–0.9) mm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cell discrete, phialidic, ampulliform, hyaline to pale brown, 3–5 × 5–7(–9) µm. Conidia hyaline when liberated, later pale brown, cylindrical to short-cylindrical, rounded at both ends, aseptate, sometimes 1-septate, thin and smooth-walled, with 2–5 oil guttules, 1–2(–3) × (7–)8–9(–10) µm.

HOST: *Huperzia selago* (L.) Bernh. ex Schrank & Mart. (*Huperziaceae*)

DISTRIBUTION: Southern Poland.

NOTES: *P. babiogorense* is most similar to *Coniothyrium lycopodinum* Sacc. & Paol., described from *Lycopodium annotinum* L. However, *P. babiogorense* conidia are longer (8–9 µm) than those of *C. lycopodinum* (6 µm), and the two species were isolated from different hosts. The short-cylindrical conidia with oil guttules of *P. babiogorense* are also similar to those of *P. cyclothyrioides* (Verkley et al. 2004). However, *P. babiogorense* conidia are larger (1–2 × 8–9 µm) and have more (2–5) oil guttules than *P. cyclothyrioides*. The SSU and ITS phylogenies also support *P. babiogorense* as different from *P. cyclothyrioides* (FIGS. 1 & 2).

Discussion

The signature sequence, low ITS sequence similarity to other *Paraconiothyrium* representatives (95%), distinct morphological characters, and monophyly support the *Huperzia selago* isolates as representing a new taxon, described above as *P. babiogorense*. The new species fits well in the concept of the

TABLE 1. Comparison of morphological characters of different *Paraconiothyrium* species.

SPECIES	REFERENCE	CONIDIOMA TYPE, DIAM. (mm)	OSTIOLE	CONIDIOGENOUS CELL SHAPE, SIZE (µm)	CONIDIA		
					# CELLS	POLAR GUTTULES	SIZE (µm)
P. babiogorense	**present study**	**eustromatic, 0.3–0.5**	**absent**	**ampulliform, 3–5 × 5–7**	**1-celled**	**2–5**	**1–2 × 8–9**
P. sporulosum	Sivanesan 1984	pycnidial, < 0.26	ostiolate	ampulliform to globose	1 celled	absent	2.5 5 × 1.5 2.5
P. minitans	Campbell 1947	pycnidial, 0.15 0.6	central	ovoid to ampulliform	1 celled	absent	4 7 × 3 4
P. brasiliense	Verkley et al. 2004	eustromatic, 0.5 2	absent	ampulliform to globose, 4 6 × 3.5 5	1 celled	few	3.2 4.6 × 2 3
P. variabile	Damm et al. 2008	pycnidial, 0.3 0.6	1 3	variable in shape, 2.5 5 × 3 7	1 celled	sometimes 2	3 4 × 1 2
P. africanum	Damm et al. 2008	pycnidial, 0.1 0.6	central	ampulliform to doliiform, 3 8 × 2 6	mainly 2 celled	absent	6.5 9.5 × 3 4
P. hawaiiense	Crous & Groenewald 2006	pycnidial, < 0.5	no data	ampulliform to subcylindrical, 5 15 × 6 7	2 celled	absent	12 13 × 5
P. cyclothyrioides	Verkley et al. 2004	eustromatic, 0.3 1.2	absent	ampulliform to subcylindrical, 4.5 8.0 × 2.5 4.0	1 celled	1 2	3 4.8 × 1.2 1.6
P. fungicola	Verkley et al. 2004	eustromatic, 0.3 1	papillate, oozes dark droplets	subglobose, or ampulliform, 5 7 × 3 5	1 celled	few	4.4 6.2 × 3 3.7
P. estuarinum	Verkley et al. 2004	eustromatic, 0.2 0.5	absent	ampulliform to subcylindrical, 4 6.5 × 2.5 3.5	1 celled	1 2	3.2 4.2 × 1.4 2

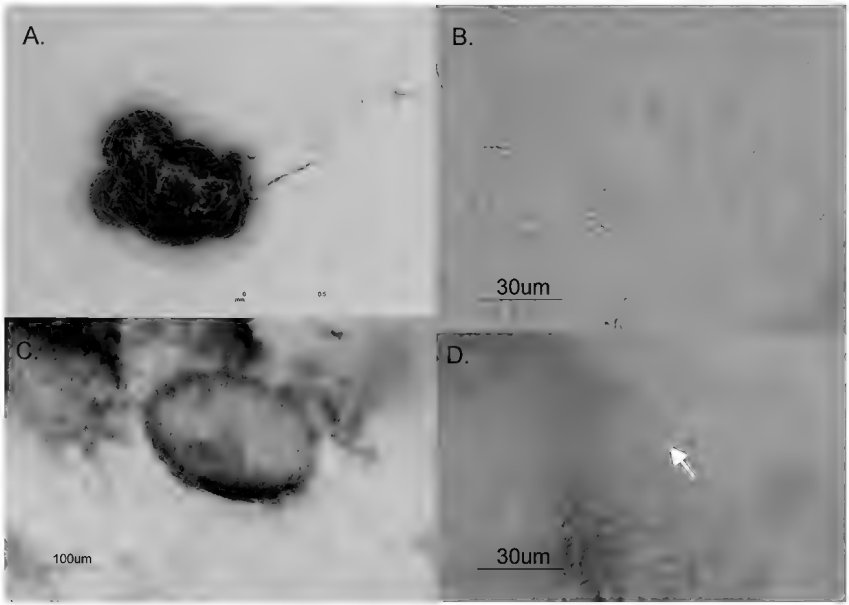

PLATE 1. *Paraconiothyrium babiogorense*: a. complex conidiomata immersed in agar medium; b. conidia; c. longitudinal section of conidiomata; d. conidiomatal wall (arrow indicates phialidic cell).

genus by the presence of eustromatic, complex conidiomata, discrete, phialidic conidiogenous cells, and thin-, smooth-walled, hyaline (when liberated) to brownish spores (Verkley et al. 2004). DNA sequence data from *P. babiogorense* also support this species as representing *Paraconiothyrium*.

Fungal endophytes of *Huperzia selago* are poorly known. Budziszewska & Szypuła (2010) identified 13 endophytic species in *H. selago* shoots from different sites, of which only two were cited by Higgins et al. (2007), who also worked on this plant. Surprisingly, neither Higgins et al. (2007) nor Budziszewska & Szypuła (2010) isolated *Paraconiothyrium* species from fir club moss shoots when our study isolated *P. babiogorense* from the vegetative reproductive structures. However, the species could have been overlooked in previous research, as it is an extremely slow growing fungus that is easily overgrown by other, faster growing ones. Thus, the presence of a new *Paraconiothyrium* species in *H. selago* shoots should be re-examined.

Although *Paraconiothyrium* species are relatively common (Damm et al. 2008), this is the first report of one isolated from fir club moss. Interestingly, *Coniothyrium lycopodinum* (Saccardo 1892: 267–268), described from leaves of *Lycopodium annotinum* from a mountain forest in Siberia, is morphologically

similar to our *P. babiogorense* isolates, at least according to its diagnosis. Moreover, two *Paraconiothyrium* sp. isolates, which phylogenetic analysis indicate are the closest relatives of the isolates from *H. selago* propagules, were also isolated from the Polish club mosses *Diphasiastrum tristachyum* (Pursh) Holub (EU709779) and *Lycopodium clavatum* L. (EU935219), suggesting some degree of host specificity.

As *P. babiogorense* was isolated from healthy plant tissue, it is not known whether it could be pathogenic to the host. Interestingly, periodic formation of the specific vegetative propagules at the shoot apex is a unique feature of some *Huperzia* species (Øllgaard 1987, Gola 2008). Moreover, the populations differ in their strategies of balancing generative and vegetative reproduction with extensive propagule production in mountain locations in opposition to the moderate number of propagules produced by lowland plants (Gola 2008). All *P. babiogorense* strains were isolated from these vegetative reproductive structures in alpine *H. selago* plants, suggesting that it could be vertically transmitted.

This fact can also explain the low genetic variability in ITS sequences among *P. babiogorense* isolates. In fact, although we sampled nine plant ramets, we are unable to determine how many plant genets there were. Thus, all isolated fungal strains may actually be vertically transmitted clones of the same individual and hence should not be treated as different strains. More research on *P. babiogorense* and *H. selago* population structures and genetic variability on this site are needed to understand the biology of the fungus.

Acknowledgments

The authors thank Dr Gerard J.M. Verkley and Dr Małgorzata Ruszkiewicz-Michalska for reviewing the manuscript and Dr Pedro W. Crous for his useful comments on the text. We would also like to thank Babia Góra National Park authorities for permission to undertake the research in the park. The study was supported by State Committee for Scientific Research, KBN, through Faculty of Biology, Warsaw University intramural grant, BW191105, in the year 2010.

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Additional species of *Graphis* from Maharashtra, India

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ABSTRACT—Our recent investigations of the lichen flora of Maharashtra have brought to light several new taxa in the family *Graphidaceae*. Nine species of *Graphis* with muriform ascospores are recognized of which two, *G. maharashtrana* and *G. elevativerrucosa*, are described as new to science. One new combination, *G. panhalensis* is proposed. Two species, *G. galactoderma* and *G. parilis*, are recorded from India for the first time, and *G. platycarpa* and *G. subducta* are reported for the first time from Maharashtra. *Graphis* sp. 1 is clearly distinguished from the other species, but is not formally described as new to science as the material is scanty. A revised key to all species of *Graphis* from Maharashtra is provided.

KEYWORDS—taxonomy, ascomycetes, lichenized fungi

Introduction

The Western Ghats of India form a practically unbroken mountain chain dominating the western coast of the Indian peninsula for almost 1600 km. They extend from the mouth of the river Tapti in Gujarat to Kanyakumari, the tip of South India in Tamil Nadu. The region consists of hills spreading north–south along the coast traversing the states of Gujarat, Maharashtra, Goa, Karnataka, Tamil Nadu, and Kerala and enjoys a tropical climate. The Western Ghats, which are exceptionally biodiverse and occupy a special position in the Indian subcontinent because of the interesting flora, are one of the richest lichen sites of India. Thus far, 800 lichen species, including 219 (i.e. 27%) potential endemics, have been recorded from this region (Singh et al. 2004).

Although a large number of lichens have been recorded from the Western Ghats of South India, Maharashtra has long been among the least explored places. During our recent investigations of the lichen flora of Maharashtra, we recorded several species in the family *Graphidaceae*, including ten species of *Graphis* with transversely septate ascospores and two species of *Dyplolabia* (Makhija et al. 2005a), ten species of *Hemithecium* (Makhija et al. 2005b; Chitale et al. 2009), three species of *Pallidogramme* (Chitale et al. 2009), and eleven species of *Diorygma* (Sharma & Makhija 2009, Makhija et al. 2009).

Graphis Adans. (sensu Staiger 2002), a widespread tropical lichen genus with about 300 species known worldwide (Lücking 2009, Lücking et al. 2009), is characterized by a thallus crustose that is corticolous and epi- or endophloeodal (rarely saxicolous or foliicolous); a green algal photobiont (*Trentepohlia*); lirellate ascomata that are usually elongate, simple or variously branched (rarely short oryzaeform), and immersed in the thallus or emergent and sessile; a totally or partially but distinctly carbonized exciple with well developed, convergent, and entire or crenate-sulcate labia; simple paraphyses that are not thickened at apices; asci clavate to subcylindrical, (1–)2–4–8-spored, unitunicate, and ascospores colourless, 3–many trans-septate, or muriform, locules lentiform, I+ blue violet.

Graphis is represented by 85 species from the Indian subcontinent (Adawadkar & Makhija 2004, 2006, 2007; Makhija & Adawadkar 2005a,b; Makhija et al. 2005a, Jagadeesh Ram et al. 2007) and by ten species with trans-septate ascospores from Maharashtra (Makhija et al. 2005a). In the present paper we report the occurrence of nine *Graphis* species with muriform ascospores from Maharashtra. Two species (*G. maharashtrana, G. elevativerrucosa*) are described as new to science. *Graphis galactoderma* and *G. parilis* are reported for the first time from India and *G. platycarpa* and *G. subducta* for the first time from Maharashtra. *Graphis panhalensis* is proposed as a new combination. *Graphis* sp. 1, which is clearly distinct from the other species, is not formally described as new to science as the material is scanty. A key is provided to all species of *Graphis* (both with trans-septate and with muriform ascospores) now known from Maharashtra,.

Materials & methods

Chemical constituents were identified by thin-layer chromatography using methods standardized for lichen products (Culberson & Kristinsson 1970, Culberson 1972, White & James 1985) with the solvent systems benzene-dioxane-acetic acid (180:45:5), hexane-ethyl ether-formic acid (130:80:20) and toluene-ethyl acetate-formic acid (139:83:8). All specimens examined are deposited in Ajrekar Mycological Herbarium (AMH).

Taxonomy

Graphis elevativerrucosa Chitale, Makhija & B.O. Sharma, **sp. nov.** FIG. 8

MycoBank MB 519296

Similis Graphis bilabiata, *sed lirellis longioribus, immerses in verrucosus et acid continens differt.*

Holotypus India. Maharashtra: Sindhudurg District, Amboli, 28.9.1976, *P.G. Patwardhan & A.V. Prabhu, 76.1233*: AMH.

ETYMOLOGY: From the Latin *elevatus* (elevated) and *verrucosus* (warty): a reference to highly elevated verruceae.

Thallus crustose, corticolous, grayish-green to dull brownish-green, distinctly warty, robust, thick; hypothallus absent. Ascomata lirelline, concolorous with the thallus, straight to curved, terminally acute, mostly simple, rarely branched irregularly, 1–9 mm long and 0.1–0.2 mm broad, immersed in verrucae of the thallus, raised; exciple, convergent, non-striate, present at the base, apically carbonized, covered with the thalline margin up to the top; hymenium hyaline, not inspersed, 84–147 µm tall, KI–; asci 2–4-spored. Ascospores hyaline, muriform, ellipsoidal, oblong, 76–109 × 25–29 µm, I+ violet.

CHEMISTRY— Constictic, stictic (major), and hypostictic (minor) acids present.

ADDITIONAL SPECIMENS EXAMINED **INDIA. MAHARASHTRA**: Sindhudurg District, Amboli, *P.G. Patwardhan & A.V. Prabhu*, 76.1258, 76.1259; *U.V. Makhija & A.V. Prabhu*, 76.1236: AMH

REMARKS— *Graphis elevativerrucosa* is well characterized by the thick, highly elevated, conspicuous warts of the thallus, apically carbonized exciple without striae; 2–4-spored asci, muriform ascospores of 76–109 × 25–29 µm and by the presence of constictic, hypostictic and stictic acids in its thallus.

Graphis elevativerrucosa shares most of the morphological characters with *G. bilabiata* Nyl., a species from Ceylon, but the latter has extremely short, sessile lirellae with a thick lateral thalline margin.

Graphis streblocarpa (Bél.) Nyl., a species with immersed to emergent lirellae, large muriform ascospores, and a smooth to subtuberculate thallus, however, differs from the present species in prominently immersed ascomata in the thalline verrucae, 1–2-spored asci, and narrow (15–30 µm diam) ascospores.

The new species has been collected in semi-evergreen forest in open places on the roadsides.

Graphis galactoderma (Zahlbr.) Lücking, Lichenologist 41: 436 (2009). FIG. 1

SPECIMENS EXAMINED **INDIA. MAHARASHTRA**: Kolhapur District, Panhala, *P.G. Patwardhan & A.V. Prabhu*, 74.1083, 74.1094, 74.1113, 74.1121; *P.G. Patwardhan & C.R. Kulkarni*, 74.1144: AMH

Thallus crustose, corticolous, epiphloeodal, uniform, continuous, pale yellow to white in colour, smooth to cracked, surrounded by thin, black hypothallus. Ascomata lirelline, up to 6 mm long and up to 0.2 mm broad, simple to branched or forked at apices, crowded, emergent to prominent, curved, wavy, flexuous, blackish, terminally acute or obtuse; lateral thalline margin not bulging, studded with crystals; exciple 1–4-striate, apically carbonized; hymenium hyaline, not inspersed, 84–100 µm tall, KI–; asci 8-spored. Ascospores hyaline, muriform, fusiform-oblong, 34–42 × 16–21 µm, I+ violet.

CHEMISTRY—Constictic, stictic (major) and norstictic acid (minor) present.

Remarks— *Graphis galactoderma* has been collected at ca. 750 m from moist open forests that get heavy rains during the monsoon. This species, earlier known from the eastern palaeotropics, is a first report from India.

Graphis insulana (Müll. Arg.) Lücking & Sipman, Fieldiana Botany 46(1): 84 (2008). Fig. 2

≡ *Graphina insulana* Müll. Arg., Engler Bot. Jahr. 4: 56 (1883).

specimens examined—**INDIA. Maharashtra**: Ratnagiri District, Nerur, *A.V. Prabhu*, 75.486, 75.487; Dabhole ghat, *A.V. Prabhu & M. B. Nagarkar*, 74.2068: AMH

Thallus crustose, corticolous, whitish-gray to light greenish-gray, smooth, cracked with the age, delimited by a black hypothalloidal region at the periphery. Ascomata lirelline, mostly immersed, to rarely slightly emergent, straight to curved, terminally acute, mostly simple, rarely irregularly branched, up to 11 mm long and up to 0.25 mm broad; exciple convergent, non-striate, laterally to sometimes completely carbonized, present at the base; hymenium hyaline, inspersed, 134–168 µm tall, KI–; asci 1-spored. Ascospores hyaline, muriform, oblong, with more or less rounded ends, 67–118 × 17–29 µm, I–.

Chemistry —Norstictic acid present.

Remarks—*Graphis insulana,* a widely distributed species in the tropical regions of the world, was earlier described from Maharashtra as *Graphina nylanderi* Patw. & C.R. Kulk. (Patwardhan & Kulkarni1979). It has also been reported from Karnataka in India.

Graphis maharashtrana Chitale, Makhija & B.O. Sharma, **sp. nov.** Fig. 3

MycoBank MB 519287

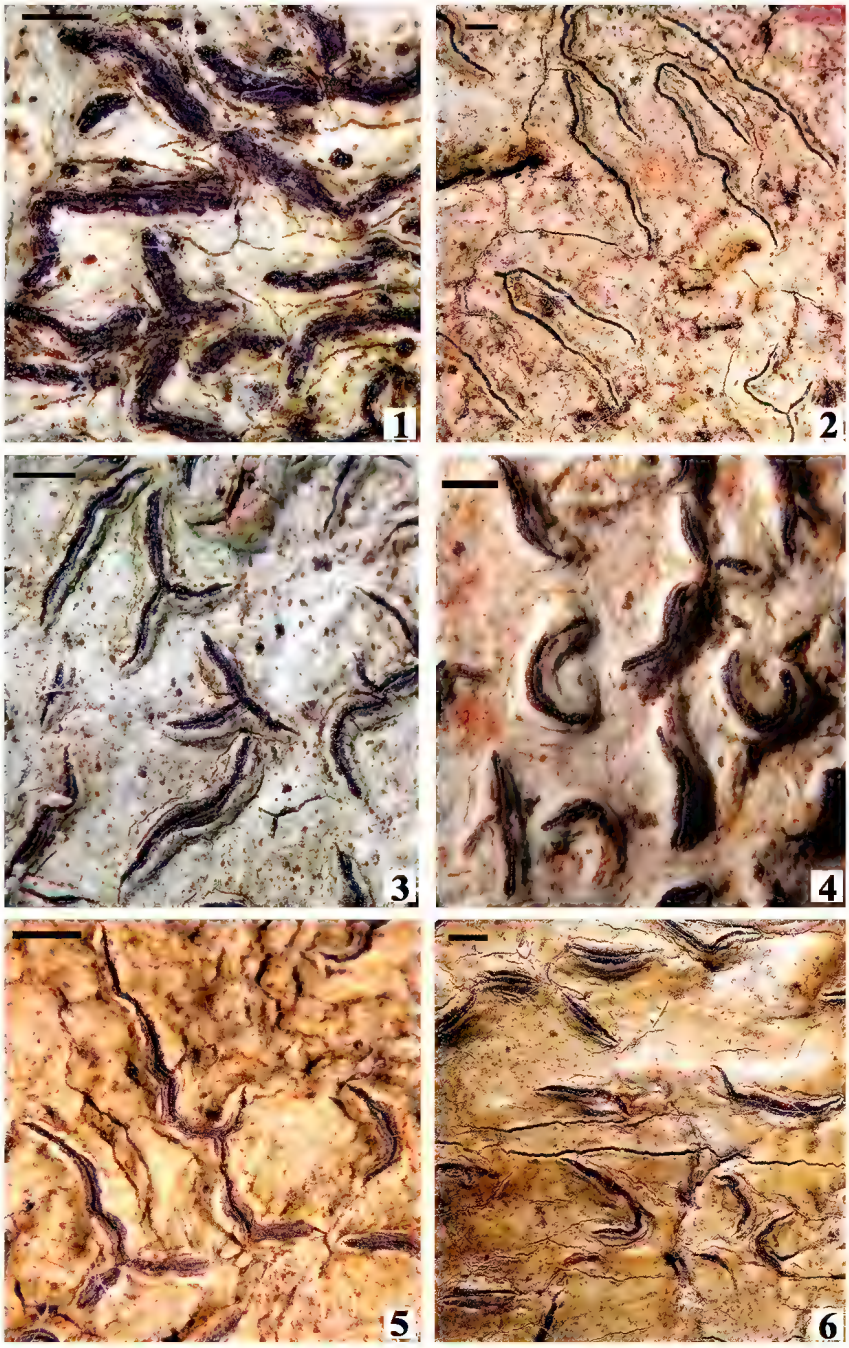
Similis Graphis panhalensis, *sed excipulo lateralis nigro, ascosporis minoribus, et acida consticticum, hyposalazinicum et sticticum continens differt.*

Holotypus—India. Maharashtra: Sindhudurg District, on the way to Ajra from Amboli, 10.10.2000, *U.V. Makhija & B.A. Adawadkar, 00.168*: AMH.

Etymology: From the state name Maharashtra.

Thallus crustose, corticolous, endophloeodal, continuous, smooth to cracked, grayish-white to dirty white, surrounded by a thin black hypothallus. Ascomata lirelline, short, up to 3 mm long and 0.1 mm broad, simple to branched, immersed to semi-emergent, irregularly curved, flexuous, scattered, concolorous with the thallus to black, terminally round to acute; exciple 4–5-striate, laterally carbonized, present at the base, convergent, covered by a thick thalline margin up to the top; studded with crystals; hymenium hyaline, not inspersed, 34–46

Figure 1 6. Habit: 1. *Graphis galactoderma* (74.1121: AMH); 2. *G. insulana* (75.486:AMH); 3. *G. maharashtrana* (Holotype); 4. *G. panhalensis* (Holotype); 5. *G. parilis* (74.1826:AMH); 6. *G. platycarpa* (74.1826: AMH). Bar 1 mm


1
2
3
4
5
6

µm tall, KI–; asci 8-spored. Ascospores hyaline, muriform, fusiform-oblong, without gelatinous sheath, 25–42 × 13–17 µm, I+ violet.

CHEMISTRY—Constictic, stictic acids present.

ADDITIONAL SPECIMENS EXAMINED **INDIA. MAHARASHTRA:** Kolhapur District, Panhala, *P.G. Patwardhan & A.V. Prabhu*, 74.1063b, 74.1076; 74.1078, 74.1091; *P.G. Patwardhan & C.R. Kulkarni*, 74.1095, 74.1101, 74.1129. Nasik District, Anjaneri, *B.C. Behera & G.S. Chitale*, 02.232; Saptashringi, *B.C. Behera & G.S. Chitale*, 02.185. Pune District, Bhimashankar, *B.A. Adawadkar*, 97.19; Lonavala, *B.C. Behera & B.A. Adawadkar*, 00.87; Khandala, *B.C. Behera & V.A. Mantri*, 00.88; Lonavala, Walvan Dam, *B.C. Behera*, 02.122, 02.123; Malshej Ghat, Neemgiri, *U.V. Makhija & A.V. Bhosale*, 02.14B; Purandar fort, *U.V. Makhija & A.V. Bhosale*, 02.55: AMH

REMARKS— *Graphis maharashtrana* belongs in a small group (Group 16, Lücking et al. 2009) of taxa with striate, laterally carbonized exciples, clear hymenium, and muriform ascospores. All species but one within this group lack lichen secondary metabolites. The exception, *G. neoelongata* Lücking, differs from the new species in having stictic and norstictic acids and very long and radiately branched lirellae.

The species can easily be differentiated from the morphologically similar *G. panhalensis* in having a laterally carbonized exciple, smaller ascospores of 25–42 × 13–17 µm and constictic and stictic acids. *Graphis panhalensis* has apically carbonized exciple and larger ascospores of 45–65 × 12–16 µm; it contains has only stictic acid.

Graphis maharashtrana has been collected from the bark of *Mangifera indica* in the dry deciduous forests.

Graphis panhalensis (Patw. & C.R. Kulk.) Chitale, Makhija & B.O. Sharma, **comb. nov.** FIG. 4

MYCOBANK MB 519294

≡ *Graphina panhalensis* Patw. & C.R. Kulk., Norw. J. Bot. 26: 47 (1979).

Thallus pale glaucous green, smooth to indistinctly warty; ascomata emergent, black, simple, curved or flexuous, 0.5–2 mm long, ends subacute; exciple 4–5-striate, carbonized only at the apices; hymenium not inspersed; asci 8-spored; ascospores hyaline, muriform, ellipsoid, 45–65 × 12–16 µm.

CHEMISTRY—Stictic acid present.

SPECIMEN EXAMINED **INDIA. MAHARASHTRA:** Panhala, 13.10.1974, *P.G. Patwardhan & C.R. Kulkarni, 74.1075*: AMH **(holotype)**

REMARKS—*Graphis panhalensis,* established as *Graphina panhalensis* by Patwardhan & Kulkarni (1979) from Maharashtra, is now placed in *Graphis* in the new system of Staiger (2002) based on its convergent, well developed exciple with distinct carbonized areas and colourless ascospores. This species is known only from its type.

Graphis parilis Kremph., Flora 59: 422 (1876). FIG. 5

SPECIMENS EXAMINED **INDIA. MAHARASHTRA**: Satara District, Mahabaleshwar, Arther Seat, *M.B. Nagarkar & A.V. Prabhu*, 74.1765, 74.1769; Lodwick point, *M.B. Nagarkar & A.V. Prabhu*, 74.1826, 74.1839, 74.1849, 74.1910, 74.1911; *A.V. Prabhu & M.B. Nagarkar*, 74.1823, 74.1824, 74.1825, 74.1827, 74.1835, 74.1872, 74.1927, 74.2952; Kolhapur District, Panhala, 13.10.1974, *P.G. Patwardhan & A.V. Prabhu*, 74.1132; Pune District, Malshej ghat, *U.V. Makhija & A.V. Bhosale*, 02.17: AMH

Thallus crustose, corticolous, epiphloeodal, continuous, smooth to cracked, or warty, greenish-yellow, surrounded by a thin, black hypothallus. Ascomata lirelline, up to 10 mm long and 0.2–0.5 mm broad, branched, curved, totally immersed to semi-emergent, or emergent, flexuous, concolorous with the thallus, terminally acute; exciple yellowish-brown, present at the base, apically carbonized, and convergent, 3–8-striate, covered by a thick thalline margin up to the top, studded with crystals; hymenium hyaline, not inspersed, 84–126(–136) µm tall, KI–; asci 2–8-spored. Ascospores hyaline, muriform, oblong, 46–76 × 13–17 µm, I+ violet.

CHEMISTRY—Stictic acid present.

REMARKS—*Graphis parilis,* a species earlier reported from Brazil, Canada, Florida, and Mexico, is here reported from India for the first time.

Graphis platycarpa Eschw., Fl. Brasil. 1:74 (1833). FIG. 6

SPECIMENS EXAMINED **INDIA. MAHARASHTRA**: Satara District, Mahabaleshwar, Arther Seat, *M.B. Nagarkar & A.V. Prabhu* 74.1759, 74.1763; Lodwick Point, *M.B. Nagarkar & A.V. Prabhu*; *A.V. Prabhu & M.B. Nagarkar*, 74.1864, 74.1902, 74.1839, 74.1910, 74.1911: AMH

Thallus crustose, corticolous, greenish-gray, epiphloeodal, continuous, smooth to cracked, uneven, rough, surrounded by a thin, black hypothallus. Ascomata lirelline, 1–3.2 mm long and 0.1–0.25 mm broad, simple to rarely with short branches, semi-emergent, scattered, concolorous with the thallus, terminally acute; exciple present at the base, 1–5-striate, carbonized only at tips, converging at the apical portion, covered by a thick thalline margin up to the top, studded with crystals; hymenium hyaline, clear, 100–136 µm tall, KI–; hypothecium hyaline to yellowish, 16–20 µm thick; asci 8-spored. Ascospores hyaline, muriform, fusiform-oblong, without gelatinous sheath, 7–14-transverse septa, lumina lenticular, 59–71 × 13–17 µm, I+ violet.

CHEMISTRY— No lichen substances present.

REMARKS— *Graphis platycarpa* was found in semi-evergreen forest in shady places. The most similar species, *G. glaucorufa* Vain., differs from *G. platycarpa* by its 1-spored asci.

Graphis platycarpa was earlier known from Manipur, India, and is reported for the first time from Maharashtra.

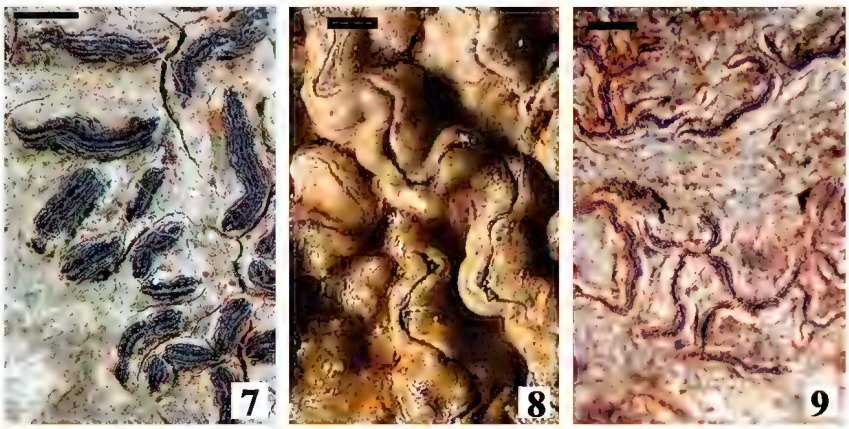


FIGURE 7 9. Habit. 7. *Graphis subducta* (04.89:AMH); 8. *G. elevativerrucosa* (Holotype); 9. *Graphis* sp. 1. Bar = 1 mm

Graphis subducta Vain., Ann. Acad. Sci. Fenn., ser. A, 15(6): 203 (1921). FIG. 7

SPECIMENS EXAMINED **INDIA. MAHARASHTRA:** Kolhapur District, Panhala, *P.G. Patwardhan & A.V. Prabhu*, 74.1085, *G.S. Chitale*, 02.282. Ratnagiri District, Chiplun, *G.S. Chitale*, 04.89: AMH

Thallus crustose, corticolous, grayish, flaking, finely cracked, smooth to rough; hypothallus black. Ascomata lirelline, simple to rarely branched, triradiate, curved, emergent, up to 1.5 mm long, 0.2–0.5 mm broad; thalline margin raised, concolorous with the thallus; exciple convergent, 3–4-striate, apically carbonized; hymenium hyaline, not inspersed, 75–90 µm tall, KI+ blue-violet; asci 8-spored. Ascospores hyaline, muriform, small, ellipsoidal, 24–33 × 9–12 µm, I+ blue-violet.

CHEMISTRY— Constictic and stictic acids present.

REMARKS— *Graphis subducta,* a species previously known from the Philippines and in India from Nagaland, is here reported for the first time from Maharashtra.

***Graphis* sp. 1** FIG. 9

SPECIMEN EXAMINED **INDIA. MAHARASHTRA:** Satara District, Mahabaleshwar, Lodwick point, *M.B. Nagarkar & A.V. Prabhu*, 74.1876, 74.1909: AMH

Thallus crustose, corticolous, epiphloeodal, continuous, smooth to cracked, uneven, slightly rough, greenish-yellow, surrounded by thin, black hypothallus. Ascomata lirelline, 1.5–7 mm long and 0.1–0.6 mm broad, simple to sparsely branched, semi-emergent, irregularly curved, flexuous, intricate, concolorous with the thallus, terminally acute; exciple 1–5-striate, yellowish-brown,

carbonized at apices, convergent, covered by a thick brown to blackish thalline margin up to the top, studded with crystals; hymenium hyaline, not inspersed, 126–176 µm tall, KI–; asci 8-spored. Ascospores hyaline, muriform, elliptical-oblong, 63–101 × 17–21 µm, I+ violet.

CHEMISTRY —Stictic acid present.

REMARKS—The present species is distinct in having unusual combination of large ascospores and chemistry. It is similar to *Graphis parilis* in respect of morphological characters and chemistry. However, *G. parilis* has much smaller ascospores of 46–76 × 13–17 µm. Since our material is rather scanty, we do not propose a new species.

Key to the species of *Graphis* from Maharashtra

1a. Ascospores trans-septate 2
1b. Ascospores muriform 11

2a. Proper exciple partially carbonized 3
2b. Proper exciple completely carbonized including at the base 10

3a. Proper exciple apically carbonized 4
3b. Proper exciple laterally carbonized down to the base 5

4a. Thallus verruculose; ascomata black, 0.5–4 mm long; exciple present below, 3–4 striate; ascospores 21–39 × 4–7 µm; stictic acid present.. ***G. vittata*** Müll. Arg.
4b. Ascomata concolorous with the thallus, 0.5–6 mm long, more or less effuse; exciple entire to 2–4 striate, indistinctly present at the base; ascospores 21–49 × 4–6 µm; constictic, connorstictic, and norstictic acids present ..... ***G. exalbata*** Nyl. (*G. nerurensis* Makhija et al.)

5a. Exciple striate [Ascomata 0.1–7 mm long, black; exciple 2–13-striate, partially to completely carbonized; ascospores oblong, 12–24-trans-septate, with one end acute, 25–109 × 4–8 µm; no lichen substances present.] ..... ***G. polystriata*** Makhija et al.
5b. Exciple not striate 6

6a. Norstictic acid absent [Ascomata 1–8 mm long, black; exciple present at base, ascospores 5–8-trans-septate, 20–38 × 5–8 µm; constictic and stictic acids present ..... ***G. modesta*** Zahlbr.
6b. Norstictic acid present 7

7a. Ascospores less than 50 µm long 8
7b. Ascospores more than 50 µm long 9

8a. Thallus smooth to minutely warty; ascomata 1–4 mm long, black; exciple present at base; ascospores 9–11-trans-septate, (20–)30–40 × 6–8 µm; constictic, norstictic and stictic acids present ..... ***G. ajarekarii*** Patw. & C.R. Kulkarni
8b. Thallus smooth to plicate; ascomata 1–2.5 mm long, black; exciple present below; ascospores 3–10-trans-septate, 17–42 × 5–9 µm; constictic and norstictic acids ..... ***G. librata*** C. Knight

9a. Thallus brownish to greenish-grey; ascomata 0.5–6 mm long, concolorous with the thallus; exciple may or may not be present at the base; ascospores 8–16-trans-septate, 25–60 × 5–11 µm; norstictic acid present ..... ***G. pyrrhocheiloides*** (Vain.) Zahlbr.

9b. Thallus whitish; ascomata 1–8 mm long, black; exciple present at base; ascospores 8–11-trans-septate, 25–50 × 7–10 µm; norstictic and stictic acids present ..... ***G. cincta*** (Pers.) Aptroot (*G. guimarana* Vain.)

10a. Thallus off white; ascomata 1–5 mm long, black; exciple present at base, multistriate; ascospores 6–9(–12)-trans-septate, 21–35 × 4–8 µm; no lichen substances present ..... ***G. duplicata*** Ach.

10b. Thallus olivaceous buff to citrine green; ascomata 0.5–3 mm long, black; exciple present at base, 3–4-striate; ascospores 7–10-trans-septate, 30–45 × 6–10 µm; no lichen substances present ..... ***G. aurita*** Eschw. (*G. persulcata* Stirt.)

11a. Exciple laterally carbonized 12

11b. Exciple apically carbonized 13

12a. Ascomata immersed, to slightly emergent, 0.1–11 mm long; exciple sometimes completely carbonized, without striae; asci 1-spored; ascospores 67–118 × 17–29 µm; norstictic acid present ..... ***G. insulana***

12b. Ascomata grayish-white to dirty white to blackish, 0.1–3 mm long, immersed to semi-emergent; exciple 4–5-striate; ascospores 25–42 × 13–17 µm; constictic, stictic and hyposalazinic acids present ..... ***G. maharashtrana***

13a. Exciple not striate. [Ascomata emergent, 1–8 mm long; asci 2–4-spored; ascospores 76–109 × 25–29 µm; constictic, hypostictic (trace) and stictic acids present] ..... ***G. elevativerrucosa***

13b. Exciple striate 14

14a. Ascospores less than 50 µm long 15

14b. Ascospores more than 50 µm long 16

15a. Ascomata up to 6 mm long. Thallus pale yellow to white; ascomata 1–6 mm long, simple to branched, semi-emergent to emergent; exciple, 1–4-striate; ascospores 34–42 × 16–21 µm; constictic, norstictic (trace) and stictic acids present ..... ***G. galactoderma***

15b. Ascomata simple to rarely branched, tri-radiate, curved, emergent, 1–1.5 mm long; exciple 3–4-striate; ascospores 24–33 × 9–12 µm; constictic and stictic acids present ..... ***G. subducta***

16a. Lichen substances absent. [Ascomata 1–3 mm long, simple, or rarely with short branches, semi-emergent; exciple 1–5 striate; ascospores 59–71 × 13–17 µm] ..... ***G. platycarpa***

16b. Lichen substances present 17

17a. Ascospores up to 100 µm long. [Ascomata 1.5–7 mm long, simple to sparsely branched, semi-emergent; exciple 1–5-striate; ascospores 63–101 × 17–21 µm; stictic acid present] ..... ***Graphis*** sp. 1

17b. Ascospores less than 100 µm long 18

18a. Ascomata concolorous with the thallus, 1–10 mm long, branched, totally immersed, semi-emergent, or emergent; exciple yellowish-brown, 3–8-striate; ascospores 46–76 × 13–17 µm; stictic acid present ..... ***G. parilis***

18b. Ascomata black, short, 0.5–2.0 mm long, emergent; exciple 4–5-striate; ascospores 45–65 × 12–16 µm; stictic acid present ..... ***G. panhalensis***

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Septobasidium sichuanense sp. nov. (*Septobasidiaceae*) from China

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Abstract — A new species, *Septobasidium sichuanense*, is described. It was collected from Sichuan Province, China.

Key words — *Pucciniomycetes*, *Septobasidiales*, taxonomy

A species of *Septobasidium*, recently collected from Sichuan Province, is described here as new:

Septobasidium sichuanense S.Z. Chen & L. Guo, **sp. nov.** Figs. 1–7

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Basidiomata resupinata, 0.4 6.5 cm longa, 0.3 4 cm lata, alba vel cinnamomeo-brunnea, margine determinata, superficie laevia, in sectione 240 1300 µm crassa. Subiculum brunneum, 15 40 µm crassum. Columnae brunneae, primum 30 60 µm altae, deinde 120 290 µm altae, 15 250 µm latae. Strata hyphararum 40 620 µm alta. Interdum hyphae repullulantes, super hymenium stratum hypharum secundum 410 650 µm altum formantes. Hymenium 45 90 µm crassum. Basidia cylindrica vel clavata, recta vel curvata, 2-cellularia, 17 27 × 6 7.5 µm, hyalina vel brunneola. Sine probasidio. Basidiosporae non visae. Haustoria ex cellulis globularibus et hyphis irregulariter spiralibus constantia.

Type: On *Punica granatum* L. (*Punicaceae*): China, Sichuan, Mianning, alt. 1873 m, 17.IX.2010, Y.F. Zhu & L. Guo 368, HMAS 242046 (**holotype**), associated with *Lepidosaphes* sp. (*Diaspididae*).

Basidiomata on trunks and branches, resupinate, 0.4–6.5 cm long, 0.3–4 cm wide, patches frequently confluent, white or cinnamon-brown; margin determinate, surface smooth. In section 240–1300 µm thick. Subiculum brown, 15–40 µm thick. Pillars brown, 30–60 µm high in the young stage and 120–290 µm high in the older stage, 15–250 µm wide. Hyphal layer 40–620 µm high. Hymenial layer 45–90 µm thick, sometimes from hymenial layer the fungal hyphae renew growth to form a second layer, 410–650 µm high. Basidia arising

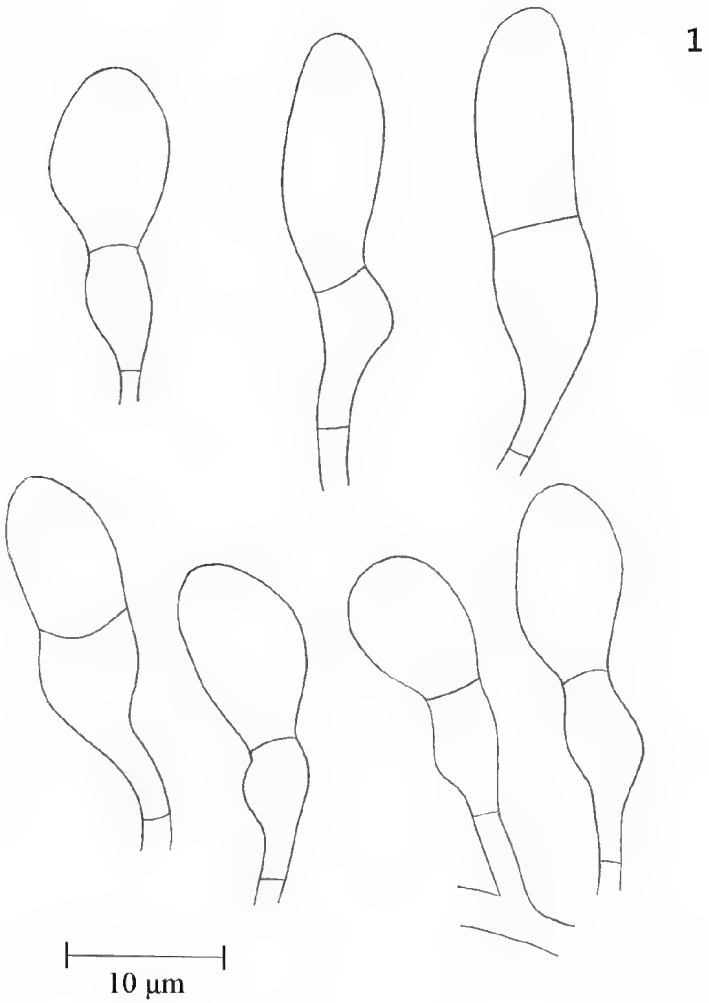
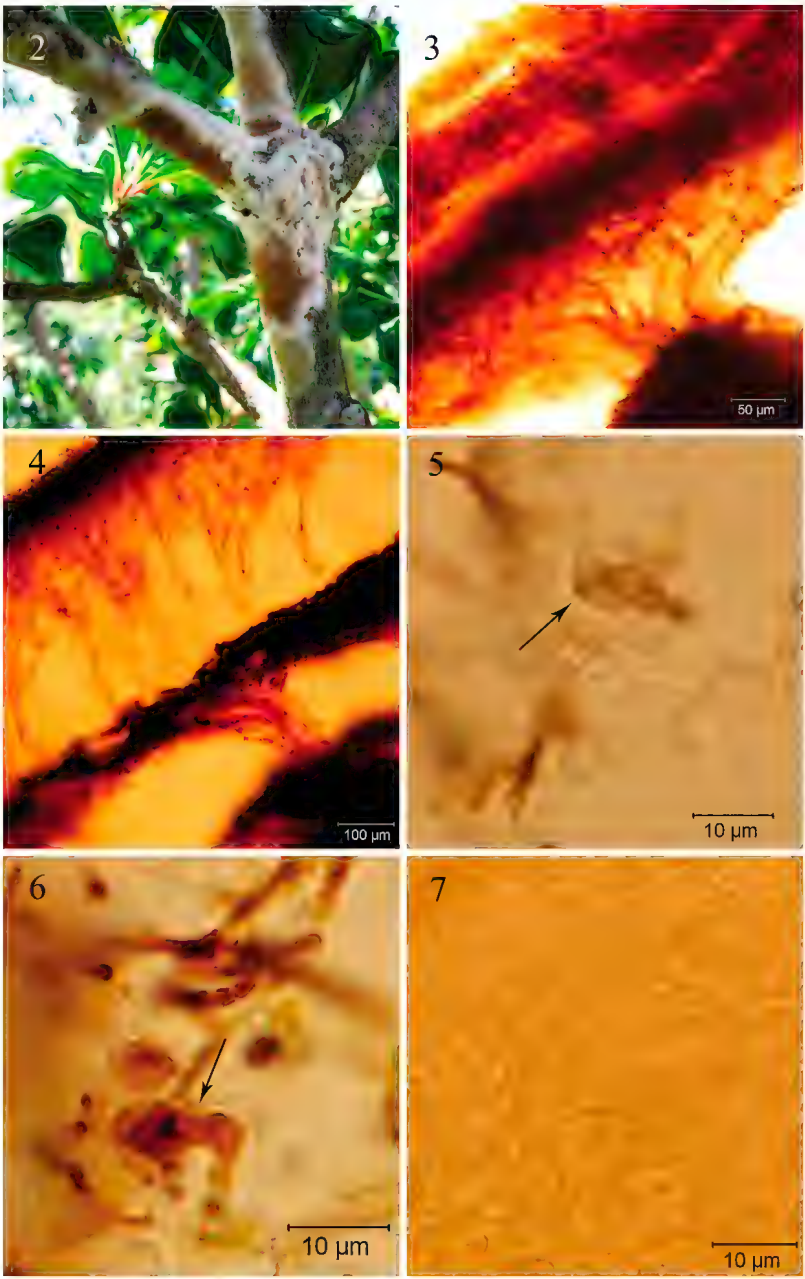


Fig. 1. Basidia of *Septobasidium sichuanense* (HMAS 242046, holotype).

directly from the hyphae without a probasidial cell, cylindrical or clavate, often constricted at the septum, straight or curved, 2-celled, 17–27 × 6–7.5 μm, hyaline or brownish. Basidiospores not seen. Haustoria consisting of both globose cells and irregularly coiled hyphae, hyaline.

Additional specimen examined: On *Cotoneaster franchetii* Bois (*Rosaceae*): China, Sichuan, Yanyuan, Mianya, alt. 3164 m, 13.IX.2010, Y.F. Zhu & L. Guo 322, HMAS 250986.



FIGS. 2–7. *Septobasidium sichuanense* (HMAS 242046, holotype).
2. Basidiomata on trunk. 3–4. Sections of basidiomata. 5–6. Basidia (arrows). 7. Haustoria.

REMARKS: Morphologically, *Septobasidium sichuanense* is similar to *S. patouillardii* Burt in having 2-celled basidium but differs in forming rather regular patches of basidiomata with a smooth surface and not cracked in the older parts, lacks a purplish tint, and has thick pillars. *Septobasidium patouillardii* forms irregular patches with cracks in the older surfaces, is velvety with a purplish tint, and has slender pillars, 20–54 μm wide and in section is 300–460 μm thick.

To date, 29 species of *Septobasidium* have been reported in China (Sawada 1933, Couch 1938, Teng 1963, Tai 1979, Kirschner & Chen 2007, Lu & Guo 2009a,b,c, 2010a,b,c, Lu et al. 2010), including the new species reported in this paper.

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Hypogymnia irregularis (*Ascomycota: Parmeliaceae*)— a new species from Asia

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Abstract — *Hypogymnia irregularis* is newly described from southwest China, Nepal, Japan, and Taiwan. Similar in growth form to *H. vittata*, *H. irregularis* differs in always lacking soredia and having staggered, lateral, or almost randomly located perforations in the lower surface. In contrast, *H. vittata* usually produces soredia and has perforations more centered in the lower surface and axils. *Hypogymnia vittata* is known from Asia, North America, Central America, and Europe, while *H. irregularis* is endemic to Asia. *Hypogymnia irregularis* is postulated as being the fertile species that is the closest living relative to the sorediate *H. vittata*. Another Asian species, *H. stricta*, can be similar in appearance to *H. irregularis* and *H. vittata*, but can be differentiated both morphologically and chemically from those two.

Key words — *Lecanorales*, lichenized ascomycetes, lichenized fungi, Yunnan Province

Introduction

Since the last checklist of lichens in China (Wei 1991), numerous species of *Hypogymnia* from Asia have been described or revised (Chen 1994, Elix & Jenkins 1989, McCune et al. 2002, McCune & Obermayer 2001, Sinha & Elix 2003, Tchabanenko & McCune 2001, Wei & Bi 1998, Wei & Wei 2005, Wei et al. 2010). But still this center of diversity for *Hypogymnia*, particularly in southwestern China, holds additional undescribed species. This paper describes a species that can be one of the dominant lichens in the high-mountain *Abies* forests of southwest China and also occurs in Nepal, Taiwan, and Japan. The species has not previously been described because of its similarity to esorediate *H. vittata* and the recently resurrected *H. stricta* (Hillmann) K. Yoshida (Yoshida & Kashiwadani 2001). The new species was, however, keyed in McCune (2009) as "*H.* sp. undescribed, close to *H. vittata*."

Methods

Standard microscopy and chemical spot test methods were applied to over 600 specimens of *H. vittata* and closely related species, from throughout its range, including

417 specimens of *H. vittata* s. str. and 149 specimens of the new species described here. A total of 86 specimens belonging to the new species, 20 of *H. stricta*, and 92 of *H. vittata* s. str. were subjected to thin-layer chromatography (TLC), using standard methods of Culberson et al. (1981). Fragments of specimens were extracted in acetone at room temperature, spotted on aluminum-backed silica gel plates (Merck 5554/7 Silica gel 60 F_{254}), run in solvent systems A and C of Culberson et al. (1981), lightly brushed with 10% H_2SO_4, and charred in an oven at 100°C.

Taxonomy

Hypogymnia irregularis McCune, **sp. nov.** FIGURES 1, 2A–D

MYCOBANK MB 519394

Ab Hypogymnia vittata *differt sorediis absentibus, ascosporis 5–6 × 4–5 µm; foraminibus infernis irregularibus.*

TYPE: China: Yunnan Province, Jiaoxi Mountain, north of Kunming, 26.100°N 102.867°E, 3700 m, *Abies georgei* var. *smithii*–*Rhododendron* forest on slopes near hotel, on bark of *Abies*, Sept. 2000, *McCune 25576* (**holotype:** KUN; **isotypes:** H, HMAS-L, OSC, UPS, US)

ETYMOLOGY: *irregularis*, referring to the irregular arrangement of perforations through the lower surface.

THALLUS appressed to pendulous, to 10(–30) cm long, branching variable, including both isotomic and anisotomic portions, lateral budding often present; texture cartilaginous rather than papery; upper surface white to pale greenish gray, sometimes with dark mottles, sometimes with black borders, smooth to weakly rugose, epruinose; lobes 0.5–3(–4) mm wide separate to imbricate, often arcuate-recurved, width even to slightly expanding distally and near the nodes, sometimes ± pinched at the nodes; lobe width:height ratio 0.5:1 to 2:1; perforations present on many lobe tips, axils, and lower surfaces, the holes not rimmed; medulla hollow, both the ceiling and floor of the cavity soon darkening to brown or dark brown; soredia and isidia lacking; lateral budding or lobules occasional.

APOTHECIA common, substipitate to stipitate, to 9 mm broad, the receptacle urn or funnel shaped; stipe hollow; disk brown; spores small, subglobose, 5–6 × 4–5 µm; pycnidia common, brown to black; spermatia weakly bifusiform, 5–6 × 0.5–0.7 µm; photobiont chlorococcoid.

CHEMISTRY — By TLC the thallus contains atranorin and physodic acid, usually with 3-hydroxyphysodic and/or vittatolic acids (all four substances present in the holotype); cortex K+ yellow; medulla K– or K+ slow reddish brown, KC+ orange-red, P–. Of 86 specimens with TLC results, all had both atranorin and physodic acid, 70% had 3-hydroxyphysodic acid, and 91% had vittatolic acid. No attempt was made to distinguish atranorin from chloroatranorin.

SUBSTRATE — On bark and wood of both conifers (especially *Abies*, *Picea*, *Sabina*, *Pinus*, and *Tsuga*, in order of decreasing frequency) and hardwoods

FIG. 1. *Hypogymnia irregularis* habit and perforations. Arrows indicate examples of dorsal (D), lateral (L), and ventral (V) perforations (McCune 25576, holotype). Dorsal perforations are infrequent, lateral perforations occasional, and ventral perforations common. Scale bar 1 mm.

(especially *Rhododendron*, *Quercus*, *Sorbus*, and dwarf bamboo).

DISTRIBUTION — Very common and often locally abundant in southwest China, especially Yunnan and Sichuan Provinces; infrequent disjuncts in India,

Nepal, and Taiwan. In southwest China, where the species is most abundant, it occurs primarily between 3000 and 4400 m in elevation, in *Abies–Rhododendron* forests above the zone of hardwood dominance.

BRIEF CHARACTERIZATION — Lobes free, short or long, often arcuate-tipped; long, slender, adventitious lobes often abundant; lobes 0.5–4 mm broad and internodes often 1–2 cm long; lobes somewhat pinched and swollen or smooth in profile; ceiling of cavity grayish brown to black; floor brownish black; similar to *H. vittata* but no soredia and perforations on lower surface irregularly arranged or staggered and partly lateral.

SELECTED SPECIMENS EXAMINED **CHINA. SICHUAN PROVINCE:** Er Mei Mt., 29.5°N 103.33°E, 2100 m, *Tao De Din 1415* (KUN); from Ping Wu Co to Jui Zhai Guo, 29.53°N 104°E, 3100 m, *Wang Li-song 86-2511a* (KUN); XINXING CO.: Gongga Mt, Yen Zi Village, 29.67°N 102°E, 3350 m, *Xuan Yu 1378* (KUN); KANGDING CO. (as Sikang: Kangting (Tachienlu), montes orientalis), *Harry Smith, s.n.* (UPS); LUDING CO.: Gongga Mt, Yen Zi Village, 29.67°N 102°E, 3250 m, *Xuan Yu 1377* (KUN); Gongga Mt., Hai Luo Gou, 29.833°N 102.33°E, 2450 3000 m, *Wang Li-song 96-16111, 96-17030, 96-16295b, 96-16213* (KUN); Gongga Mt, 29.42°N 101.83°E, 3000 m, *Wang Li-song 96-17242* (KUN); MI YI CO., Ma Long Village, Bei Puo Mt., 26.83°N 102°E, 3100 3600 m, *Wang Li-song 83-775 , 83-798, 83-829* (KUN); MULI CO., E slopes of Ning Lang Mt., 27.83333°N 101.1667°E, 3900 m, *Yuan Yu 1845* (KUN); Ning-lang village, 27.23°N 100.87°E, 3900 m, *Xuan Yu 4268b* (KUN); from Yi Qu to Wo Ya, 27.83°N 101°E, 2700 3500 m, *Wang Li-song 83-2364, 83-2365* (KUN); W slope of Ning Lang Mt., 27.83°N 101.17°E, 4000 m, *Xuan Yu 1798* (KUN); Ya Zhuei forest sta., Jiang Du, 27.83°N 101.33°E, 3450 m, *Wang Li-song 83-1580* (KUN); Yi Qu, 27.83°N 101°E, 3200 3700 m, *Wang Li-song 83-2341, 83-2400* (KUN); NANPING CO., Jiu Zhai Gou, 33°N 103.83°E, 2050 3200 m, *Wang Li-song 86-2725,86-2627, 86-2660* (KUN); WENCHUAN CO.: Wo Long Nature Preserve, 30.9°N 103.1°E, 3200 m, *Wang Li-song 96-17683* (KUN); YANYUAN CO., Beiling Village, Si Da Unit, 27.5°N 101.5°E, 3500 3800 m, *Wang Li-song 83-1435, 83-1464* (KUN); Daling Village, Huo Lu Mt., 27.5°N 101.5°E, 3500 4150 m, *Wang Li-song 83-1149, 83-1175a, 83-1196, 83-1211, 83-1258, 83-1398* (KUN); **XIZANG (TIBET):** CHAYU CO.: Cawarong, Song Ta Xue Mt, 28.67°N 97.83°E, 3500 m, *Wang Li-song 82-801, 82-805* (KUN); Gyala Peri N, W above Gyala Peri-N Glacier, 29.9°N 94.87°E, 3820 m, *Miehe, G. & S. 94-215-42/10* (GZU). **YUNNAN PROVINCE:** CAOJIAN CO., Zi ben Mt, 25.737°N 99.058°E, *Wang Li-song 00-18901, 00-18910, 00-18913* (KUN); DALI CO.: Xiao Lin Feng, 25.717°N 100.13°E, *Wang H.-c. 4825b* (KUN); Long-quan-feng Mt., 25.2°N 100.95°E, *Wang Han-che 4364* (KUN); top of Long Quan Feng Mt., 25.717°N 100.133°E, *Wang H.-C. 1067, 4360* (KUN); Yu Jiu Feng, 25.63°N 100.1°E, 3000 m, *Sauer 30.154b* (KUN); trail to Mt. Cang, 25.685°N 100.102°E, 3500 m, *McCune 26772, 26775, 26779, 26780, 26787, 26803, 26806, 26795, 26797* (OSC); DEQIN CO.: Bei Ma Xue Shan, Ya Kou, 28.383°N 99°E, 4300 m, *Wang Li-song 93-13489* (KUN); Meilishi village, Suola Ya-Kou, 28.63667°N 98.61333°E, 4000 4750 m, *Wang Li-song 00-19742, 00-19766* (KUN); Mei Li Xue Mt, Xio-Nang Village, 28.4°N 98.75°E, 3400 m, *Wang Li-song 94-15041* (KUN); FU GONG CO.: Lu Ma Den Village, Qu Lu Di unit, 27.033°N 98.883°E, 3500 m, *Wang Li-song 82-448* (KUN); GONG SHAN CO., Binzhong Luo to Tong Da Ya Kou, 93.683°N 28.092°E, 3800 m, *Wang Li-song 99-18510* (KUN); Qinatong, Songtaxue Mt., 28.1883°N 98.5317°E, 3200 m, *Wang Li-song 00-19613, 00-19615, 00-19619, 00-19628* (KUN); Yen Niu Gu, 27.801°N 98.825°E, 2950 m, *Wang Li-song 00-19364, 00-19377* (KUN); Binzhong Luo village, 27.96667°N

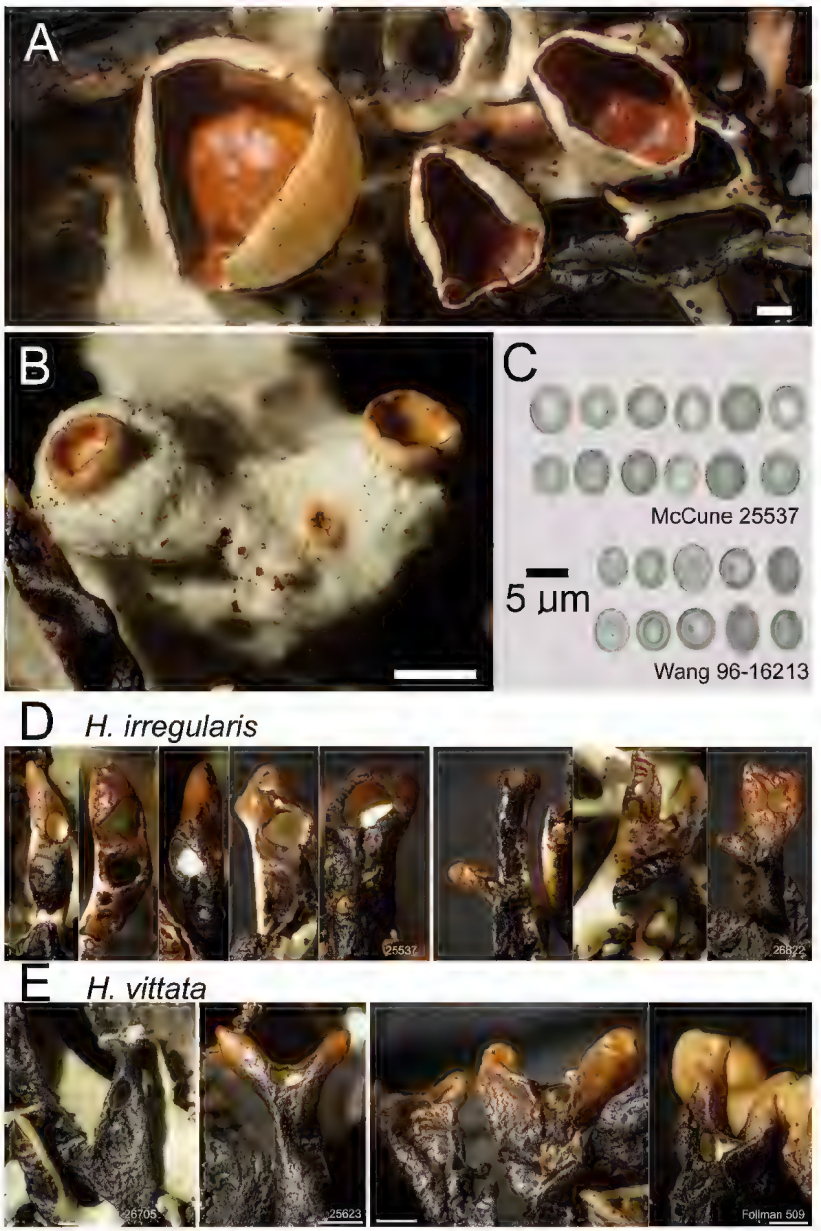

FIG. 2. *Hypogymnia irregularis*. A. Apothecia (McCune 25576; scale bar 1 mm.); B. Young apothecia (McCune 25576); C. Ascospores (Wang 96-16213; McCune 25576). D, E. Comparison of position of perforations in the lower surface of *Hypogymnia irregularis* (D) and *H. vittata* (E). Collection numbers are indicated below photos.

98.51667°E, 3600 m, *Wang Li-song 82-741* (KUN); JIANCHUAN CO.: ridge on trail to Laojun Shan, 26.6317°N 99.7183°E, 3980 m, *McCune 26728, 26729* (OSC); San Jiang Bin Liu area, 26.6625°N 99.7217°E, 3110 m, *McCune 26703* (OSC); trailhead to Laojun Shan, 26.6322°N 99.7253°E, 3700 3800 m, *McCune 26707, 26715* (OSC), *Wetmore 88456* (MIN); LIJIANG CO.: Jiushijiulongtiam Lake, Maan Mt., 26.6517°N 99.775°E, 3500 m, *Wang Li-song 00-20085, 00-20094, 00-20101, 00-20223* (KUN); Jiutte Villate, Laojun Mt., 26.65°N 99.77°E, 4000 m, *Wang Li-song 99-18732* (KUN); Laojun Mt., Jiushijiulong Lake, 26.6317°N 99.7283°E, 3800 4100 m, *Wang Li-song 00-19820, 00-20252, 00-20257, 00-20278, 00-20288, 00-20289, 00-20319, 00-20324* (KUN); Li-di-ping, 26.85°N 100.267°E, 3200 m, *Xi Jian-xun 0165, 0169a* (KUN); Yu Long Xue Mt., 27.083°N 100.167°E, *Li Xin-jiang 1494* (KUN); Lijiang area ("Likiang"), *J. F. Rock 115b* (US); eastern slopes Likiang Snow Range, *Rock,J.F. 11779* (US); LUQUAN CO., Jiaoxi Mt., 26.083°N 102.133°E, 3700 4300 m, *Wang Li-song 92-12856, 92-12857, 92-12869, 92-12879, 92-12949, 92-12950, 96-16725, 96-17060, 96-17066 Fan Rei Zhen 3220* (KUN); Jiaoxi Mt., N of Kunming, 26.100°N 102.867°E, 3500 3750 m, *McCune 25517, 25518, 25528, 25537a, 25537b, 25588, 25600, 25606, 25631, 25653* (OSC); WEI XI CO.: Li-di-ping Village, 27.167°N 99.417°E, 3200 m, *Wang Li-song 82-7* (KUN); Ye Zhi Village, Ba-ding, 27.8°N 99.03°E, 3500 m, *Wang Li-song 82-264a, Zhang D.-C. 82-83* (KUN); YUNLONG CO.: Zi Ben Shan, top of ridge, 25.733°N 99.067°E, 3200 m, *McCune, 26822* (OSC); Xiao Zhongdian, Ji Sha, 27.417°N 99.733°E, *Den Kun-Mei 81-1679* (KUN); Daxue Mt., 28.503°N 99.817°E, 3800 m, *Wang Li-song 00-19824* (KUN); Daxue Mt., Ya Kou, 28.667°N 99.834°E, 4270 m, *Wang Li-song 93-2751* (KUN); Daxue Mt., 28.567°N 99.817°E, 4400 m, *Wang Li-song 01-20740, 01-20796, 01-20800* (KUN); Daxue Mt., 28.579°N 99.821°E, 3350 m, *Watson, M. F. 18* (E); Na Pa, Hai Hou Mt., 27.833°N 99.717°E, 3860 m, *Wang Li-song 93-13742* (KUN); Tianchi (alpine lake), 27.833°N 99.717°E, 3700 m, *Wang Li-song 94-14929* (KUN); Tianchi Lake, 27.617°N 99.633°E, 3900 m, *Wang Li-song 01-20888* (KUN); Wengshuei Village, Daxue Mt., 28.575°N 99.83417°E, 4000 m, *Wang Li-song 00-20026, 00-20032* (KUN); Xiozhongdian, Tianchi, 27.833°N 99.717°E, *Wang Li-song 93-13683d, f, g* (KUN). **JAPAN. HONSHU**: Prov. Shinano, betw. Shibunoyu Hot Spring & Kuroyuridaira, 2400 m, *Kashiwadani, Lich. Rar. Crit. Exs. 478* (BM, S, US, WIS); Prov. Kai, lower slopes on N side of Mt Fuji, near Okuniwa, between 4-gome and 5-gome, 2250 m, *McCune 26062, 26069* (OSC). **NEPAL.** Rolwaling Himal: Mt. Numbur, 3400 m, *Yoda 130* (TNS); S of Kyangen Gompa, Birch wood, 28.2037°N 85.558°E, 3852 m, *Sharma et al. L10* (E). **TAIWAN**: TAICHUNG CO., Between Ssu-yuan & To-chia-tun Mt., Nanhuta Mt., 2700 m, *Kashiwadani 36156* (TNS); Nanhuta Mt., 3000 m, *Tateishi* s.n., *12 Sep 1984* (TNS); Nantou Co., Mt. Yu Shan, Tartaka Saddle to Paiyun Hostel, 3000 m, *Lai 10325* (US); Toroko NP, Hehuan Mt, 3200 m, *Mikulin T5* (OSC), 2756 m, *Mikulin T72* (OSC).

Hypogymnia vittata (Ach.) Parrique

FIGURE 2E

SPECIMENS ILLUSTRATED OR MENTIONED **AUSTRIA.** Kärnten: Gurktaler Alpen, Severgraben, E von Gnesau, ca. 800 m, 46°48'N 14°4'W, on *Alnus incana*, 27 Oct 1998, *Zeiner 151* (GZU, fertile); E Tyrol, southern foothills of Hochvenediger Mts., Follman, Lich. exs. sel. 509 (OSC). **CHINA. YUNNAN PROVINCE**: Jiaoxi Mt., north of Kunming, 26.100°N 102.867°E, 3700 m, *McCune 25623* (OSC); Jianchuan Co., San Jiang Bin Liu area, road to Lao Juen Shan, 3110 m, *McCune 26705* (OSC). **FINLAND.** Uusimaa: Espoo, Nuuksio (Nouks), Hankalahti (Hanklaks), Ahvenlampi, rock face (kallioseinällä), 21 Oct 1934 *K. Linkola* (H, fertile). Pohjois-Savo (Savonia borealis): Kuopio, Enonlahti, Enonmäki, rock face (kallion kylessä), 14 Jul 1909 *K. Linkola* (H, fertile). **MEXICO.** Estado de Oaxaca: mountain tops ca. 6 km NW of Ixtlan de Juarez, 2865 m, Esslinger 18225C (NDA).

Discussion

Hypogymnia irregularis often shows an extreme range in lobe width and length, all in the same thallus (Fig. 1), but it is generally more robust than *H. vittata*, with lobes commonly >2 mm wide and internodes >9 mm long. The arrangement of the perforations in *H. irregularis* can vary even within a single specimen from a staggered arrangement of semi-lateral holes to a linear arrangement of holes centered on the lobes, or even lateral or dorsal holes (Figs 1, 2D).

Both *Hypogymnia irregularis* and *H. stricta* (Yoshida & Kashiwadani 2001; lectotype: TNS!) appear similar to esorediate individuals of the normally sorediate *H. vittata* (type: BM! as *Parmelia physodes* var. *vittata* Ach.). Differences among these species are discussed below.

Hypogymnia stricta is currently known from China, Japan, and Taiwan. In *H. stricta* the ceiling of the lobe cavity is light brown to white (rarely dark brown), the upper cortex has faint to prominent transverse cracks, and the thallus contains 2′-*O*-methylphysodic acid (see below). In contrast, both *H. irregularis* and *H. vittata* have consistently dark ceilings, except for a whitish ceiling very near the lobe tips. The perforations of *H. stricta* are similar to *H. vittata*, being rather large and ± centered along the midline of the lower surface of the lobes, both of those differing from the irregularly placed holes in *H. irregularis* (Fig. 2D).

The ascospores of *H. stricta* average 5.5–7.0 × 4.5–5.0 µm, slightly longer than those of *H. irregularis* which are mostly 5.0–6.0 × 4.0–5.0 µm. The spore sizes of *H. irregularis* broadly overlap with *H. vittata*. Fertile specimens of *H. vittata* are rare. Two specimens from Finland had ascospores (4.8–)5.0–6.0 (–6.5) × 4.8–5.2(–5.4) µm, similar to Bitter (1901): 4.5–5.8 × 4.5–5.0 µm. A fertile specimen from Austria was barren of ascospores.

Separation of esorediate *H. vittata* from *H. irregularis* must rely on the pattern of the perforations in the lower surface and the presence and abundance of pycnidia. Although *H. vittata* typically has large, sparse perforations that are nearly centered in the lower surface or axils (Fig. 2E), *H. irregularis* has more frequent and smaller perforations that are often lateral, irregular in placement or sometimes staggered from side-to-side (Figs 1, 2D). This character is not, however, perfect. Even on a single individual of *H. irregularis*, one can observe some lobes with centered perforations and others with staggered perforations. And some specimens of *H. irregularis* have mainly centered perforations. Conversely, *H. vittata* sometimes forms perforations that deviate somewhat from the midline.

Almost always *H. vittata* has no pycnidia or only a few, widely scattered pycnidia, while *H. irregularis* usually has dense swarms of pycnidia on many lobes.

Because *H. irregularis* is restricted to Asia, it is not necessary to separate *H. vittata* from *H. irregularis* in most of the World. *Hypogymnia vittata* is known from Europe, North America, Central America (Mexico), and Asia south to New Guinea.

Some specimens from Japan are problematic. There it is common to find specimens close to *H. vittata* but without soredia and without the irregular perforations of *H. irregularis*. Perforations in the lower surface tend to be large and centrally aligned. In contrast to usual *H. vittata*, the esorediate Japanese material often has dense swarms of pycnidia, suggesting a closer affinity with *H. irregularis*. The ultimate disposition of this material requires further study; for now, it is placed in *H. irregularis*.

Hypogymnia stricta typically contains atranorin, physodic, 3-hydroxyphysodic, and 2′-*O*-methylphysodic acids (20 specimens with TLC data). Thus it has a medulla K+ slow reddish brown, KC+ orange red, and P–. *Hypogymnia irregularis* can have the same spot tests, or it can be K– when lacking 3-hydroxyphysodic acid. However, 2′-*O*-methylphysodic acids was not found in *H. irregularis* by TLC, while this substance appears to be constant in *H. stricta*.

Hypogymnia vittata is essentially the same as *H. irregularis* in frequency of substances by TLC. Of 92 specimens of *H. vittata* with TLC data (worldwide), all had both atranorin and physodic acid, 85% had 3-hydroxyphysodic acid (vs. 70% in *H. irregularis*) and 84% had vittatolic acid (vs. 91% in *H. irregularis*). Both species often show an unknown spot just under vittatolic acid in solvent C, and both commonly have long-wave UV+ unknowns in Culberson's Rf classes A5 and C6. Both species show pronounced quantitative variation in vittatolic acid, ranging from a trace (or not detected) to an obvious major substance.

The *Hypogymnia vittata* complex (Table 1) should prove a fascinating subject for molecular systematics. All species in this group have large ventral perforations that lack a raised differentiated rim, predominantly dark lobe interiors, narrow perpendicular adventitious branches, and a P– medulla (lacking physodalic and protocetraric acids). The common *H. physodes*, while similar to *H. vittata* in the soralia lining splayed open lobe tips, differs in the absence of perforations (other than those associated with soredia), the absence of narrow adventitious branches, and the P+ orange-red medulla from physodalic acid.

All species in the *H. vittata* group are Asian, except for *H. vittata*, which has spread to other continents. Like other species pairs in *Hypogymnia* (*H. lophyrea* (Ach.) Krog and *H. hultenii* (Degel.) Krog; *H. krogiae* Ohlsson and *H. incurvoides* Rass.; Miadlikowska et al. 2011), this can be considered another case of the fertile species having a narrower distribution than the sorediate counterpart.

TABLE 1. Summary of characters of *Hypogymnia vittata* and related species.

Hypogymnia species	ASEXUAL REPRODUCTION	APOTHECIA	CEILING OF LOBE CAVITY	VITTATOLIC ACID	2′ *O* METHYL PHYSODIC ACID
irregularis	none	common	dark	usually	no
pectinatula	none	not seen	dark	no?	no?
pseudobitteriana	laminal soredia, schizidia	rare	dark	seldom	no
stricta	none	common	pale	no	usually
vittata	labriform soredia	rare	dark	usually	no
zeylanica	isidia bursting into soredia	rare	dark	no	no

All have narrow perpendicular adventitious branches, are P (lacking physodalic and protocetraric acids), have non rimmed perforations in the lower surface. Only a small number of *H. pectinatula* have been tested by TLC, so chemical substances are uncertain.

In addition to the species discussed above, other apparently close relatives of *H. vittata* have a diversity of reproductive modes (Table 1): *H. pseudobitteriana* (D.D. Awasthi) D.D. Awasthi (widespread in eastern and southern Asia) has laminal schizidia and soredia; *H. zeylanica* (R. Sant.) D.D. Awasthi & Kr.P. Singh (Sri Lanka) has isidia and pustular soredia; and *H. pectinatula* (Zahlbr.) Elix (Papua New Guinea, Java, Philippines, and North Borneo) has a frondose growth form with abundant lateral budding but no other asexual propagules. All of these share with *H. vittata* a perforate lower surface, dark ceilings and floors of the lobe cavity, absence of 2′-*O*-methylphysodic, physodalic, and protocetraric acids (medulla P–), and a tendency to produce slender perpendicular adventitious branches. From this group I exclude those species with a raised, differentiated rim around the perforations (McCune et al. 2002), such as the superficially similar *H. diffractaica* McCune. Last, *H. subduplicata* (Rass.) Rass., a sorediate species described from Far East Russia, is very similar to *H. vittata*. The chemistry of the type needs to be determined and the morphology compared with *H. vittata*.

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Marasmius galbinus, a new species from China

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ABSTRACT — *Marasmius galbinus* of section *Globulares* is described and illustrated as a new species. The type is preserved in the Herbarium of Microbiology Institute of Guangdong Province (GDGM).

KEY WORDS — diversity, *Marasmiaceae*, taxonomy

Introduction

Members of *Marasmius* sect. *Globulares* possess a hymeniform pileipellis of smooth (*Globulares*-type) cells (Wilson & Desjardin 2005). There are about 100 species and varieties in this section worldwide (Singer 1976, 1986; Tan et al. 2009; Wannathes et al. 2009; Antonín et al. 2010).

Until now, no monograph of *Marasmius* has been published from China, and only 17 members of section *Globulares* have been reported from China (Dai 1979; Bi et al. 1985, 1987, 1994; Li et al. 1994; Zhang 1995; Mao 1998; Deng & Li 2008). During a survey on the Chebaling Biosphere Reserve in Shixing County of Guangdong Province, a species was collected, belonging to section *Globulares* with a unique combination of features and herein proposed as a new species.

Materials & methods

Specimens were annotated and photographed in the field, dried in an electric drier, and then preserved in herbarium. Color terms and notations follow those of Kornerup & Wanscher (1978). Fungal tissues were mounted in 5% KOH for microscopic examination. The terms used to describe lamellae spacing refer to the number of lamellae reaching from the stipe to the pileus margin and do not include the lamellulae, whose spacing is indicated by the number of series present. Spore statistics include: x_m, the arithmetic

mean of the spore length by spore width (± standard deviation) for n spores measured in a single specimen; Q, the quotient of spore length by spore width in any one spore, indicated as a range of variation in n spores measured; Q_m, the mean of Q-values in a single specimen; n, the number of spores measured per specimen; s, the number of specimens involved. Specimens are deposited in Herbarium of Guangdong Institute of Microbiology (GDGM). Authors of fungal names are cited according to the International Plant Names Index Authors website (http://www.ipni.org/ipni/authorsearchpage.do).

Genomic DNA was isolated from dried specimens and the ITS1-5.8S-ITS2 segment from the ribosomal DNA (rDNA) was amplified with primer sets ITS1 (5'-TCC GTA GGT GAA CCT GCG G -3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC -3') by polymerase chain reaction (PCR) techniques (White et al. 1990). Amplified products were examined with agarose gel electrophoresis using a 2kb DNA marker. The amplified PCR products were directly sequenced and deposited in GenBank.

Taxonomy

Marasmius galbinus T.H. Li & Chun Y. Deng, **sp. nov.** FIG. 1–2

MycoBank MB 519192

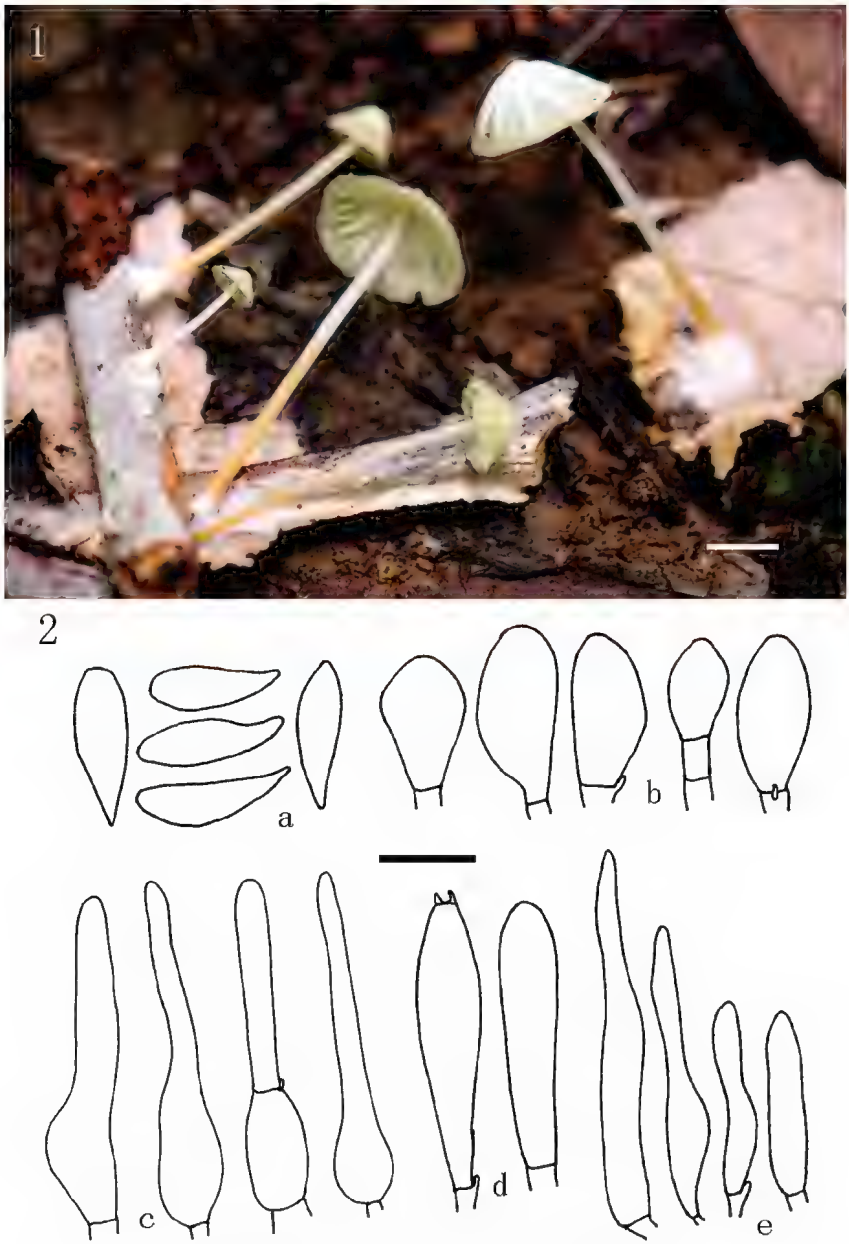
Pileus 8–15 mm latus, campanulatus, late conicus, striatus, hygrophanus, albido-luteolus. Lamellae adnexae vel subliberae, distantibus. Stipes 15–30 × 0.5–1 mm, aequalis vel subbulbosus ad basim, non-insititius, apicem albidus vel luteolus, ad basim albo-strigosus. Basidiosporae 14–16 × 4–5 µm, anguste clavatae vel sublacrymoideae, hyalinae, inamyloideis. Cheilocystidia 29–33 × 6–9 µm, clavata vel vesiculosa. Pleurocystidia nulla. Pileipellis hymeniformis (typi Globularis) ex cellulis clavato-vesiculosis, hyalinis, membrana tenuitunicata instructa. Trama pilei et lamellarum dextrinoidea. Caulocystidia 10–50 × 4–10 µm, cylindraceis, clavatis vel subfusiformibus. Trama pilei et lamellarum dextrinoidea.

Type: CHINA, Guangdong, Chebaling National Nature Reserve. 26 May 2010, Li Y.J., Huang H. & Deng C.Y. (**Holotype**, GDGM 27251).

Etymology: *galbinus* greenish yellow; referring to the basidiome color.

Pileus 8–15 mm wide, obtusely conical when young, convex to campanulate in age, rugulose at the disc, sulcate to plicate at margin, glabrous, hygrophanous, disc and striae white to greenish white (3A2), elsewhere yellowish white (2A2, 2B3). Context yellowish white, thin. Lamellae subfree to adnexed, distant (14–16) with 0–3 series of lamellulae, broad (1–2 mm), yellowish white, non-marginate, not intervenose. Stipe 15–30 × 0.5–1 mm, central, cylindrical with or without a sub-bulbous base, hollow, glabrous, non-insititious, apex hyaline to white, base pale yellow (4A3) to light yellow (4A4, 4A5). Odor and taste not distinctive.

Basidiospores 14–16 × 4–5 µm [x_m = 15.68 ± 0.7 × 4.6 ± 0.5 µm, Q = 3.2–3.4, Q_m = 3.38 ± 0.1, n = 25 spores, s = 1 specimen], clavate to fusoid, often curved in profile, smooth, hyaline, inamyloid, thin-walled. Basidia 30–35 × 6–8 µm, clavate, 2- or 4-spored. Basidioles clavate to cylindrical.



Figs 1–2: *Marasmius galbinus* (holotype, GDGM 27251).
1. Basidiomes; 2. a. Basidiospores, b. Pileipellis, c. Cheilocystidia, d. Basidia, e. Caulocystidia.
Bars: 1 – 1 cm; 2 – 10 μm.

CHEILOCYSTIDIA abundant, lamellar-edge sterile, 29–33 × 6–9 µm, irregularly clavate to ventricose, hyaline, inamyloid, thin-walled. PLEUROCYSTIDIA absent. PILEIPELLIS a hymeniform layer of *Globulares*-type cells, 12–21 × 9–12 µm, broadly clavate to pyriform, hyaline, inamyloid, thin to thick-walled, non-gelatinous. LAMELLAR TRAMA regular; hyphae 2.5–10 µm diam., cylindrical, hyaline, dextrinoid, thin-walled, non-gelatinous. STIPITIPELLIS hyphae 4–10 µm diam., parallel, cylindrical, smooth, dextrinoid, thin- to thick-walled, non-gelatinous. STIPE TRAMA hyphae 3–6 µm diam., parallel, cylindrical, hyaline, smooth, dextrinoid to weakly dextrinoid, thin-walled, non-gelatinous. CAULOCYSTIDIA 10–50 × 4–10 µm, versiform, ranging from irregular cylindrical to fusoid, clavate or irregular in outline, hyaline, inamyloid, thin- to thick-walled. Clamp connections present in all tissues.

ECOLOGY AND DISTRIBUTION—Scattered on dicotyledonous leaves or debris in broad-leaved forest in May; China (Guangdong).

COMMENTS—The main features of *Marasmius galbinus* include: a hygrophanous, striate pileus with (greenish) white disc and striae; distant to subdistant yellowish white lamellae; large basidiospores with means 15.6 × 4.0 µm; the presence of cheilocystidia and caulocystidia; and the absence of pleurocystidia.

Marasmius galbinus is similar in color to three other yellowish green species, Asian *M. grandiviridis* Wannathes et al., American *M. rhyssophyllus* Mont. ex Berk. & M.A. Curtis, and African *M. staudtii* Henn. *Marasmius grandiviridis* differs in forming a larger (37–88 mm diam.), plicate pileus and larger basidiospores (26–30 × 4–5 µm) (Wannathes et al. 2009b); *M. rhyssophyllus* differs in forming narrow, closely interveined lamellae and smaller basidiospores (5–6.5 × 3.5–4.5 µm) (Pegler 1983); and *M. staudtii* differs in forming a sulcate pileus and longer basidiospores (23–26 µm) (Singer 1964).

Macroscopically, the new species resembles the African species *M. albidocremeus* Antonín and South Pacific species *M. musisporus* Desjardin & E. Horak. *Marasmius albidocremeus* is distinct by a whitish or pale cream pileus, a long and slender stipe (50–90 × 0.5–1.25 mm), larger basidiospores (16.5–23 × 3.5–5.0 µm) and well-developed pleurocystidia (Antonin 2003), while *M. musisporus* differs by very large basidiospores (30–40 µm long) and the absence of caulocystidia (Desjardin & Horak 1997)

The rDNA-ITS (ITS1-5.8S-ITS2 segment) sequence with 657 bps of the new species (HQ709445) differs from all other known *Marasmius* sequences. Through a Blast search against the GenBank DNA database, only 160 bps of 5.8S of the sequence can be compared with 998 max scores and 95% maximal percent identities to those of *M. laticlavatus* (EU643511, EU643512), and with 929 max scores and 92% maximal percent identities to those of *M. purpureostriatus* (EU935539). The remaining parts (ITS1 and ITS2,

occupying about 75.6% of the whole segment) of the sequence are so different that they are not comparable with the known sequences. Therefore, *M. galbinus* is considered distinct.

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A new *Puccinia* on *Thymelaea* from Turkey

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Abstract — The rust fungus *Puccinia salihae* sp. nov. is described from *Thymelaea aucheri* in East Anatolia, Turkey, producing aecia, uredinia, and telia. This is the first report of a rust disease infecting a species of *Thymelaea*.

Key words — phytopathogens, *Pucciniales, Thymelaeaceae, Uredinales*

Introduction

Although many studies on the flora of Turkey have been carried out, the mycobiota is comparatively less studied (Göbelez 1963; Kırbağ 2003, 2004; Hüseyin & Kırbağ 2003; Hüseyin 2004; Bahcecioglu et al. 2009). During surveys of the parasitic fungi on herbaceous plants in the Elazıg province of Turkey a species of *Puccinia* Pers. was observed on *Thymelaea aucheri*, a perennial herb. The disease was observed on host leaves, fruit, and stems. Disease incidence was rare, detected only once during three years' observation. Very few rusts have been recorded from *Thymelaeaceae* and none from *Thymelaea* Mill. (Farr & Rossman 2010).

Materials & methods

The material for this study was collected from the Elazıg-Baskil Province in Turkey in 2007, from square B7 at an altitude of 1800 m. The typical climate of the region is Mediterranean, characterized by hot and dry summers and extremely cold and snowy winters. Field collected specimens were preserved by drying in a plant press according to established herbarium techniques. The host plant was identified using the flora of Turkey (Davis 1985). Holotype material is housed at the Firat University Herbarium (FUH) in Elazig, Turkey; isotype material is housed at the US National Fungus

Collections, Beltsville, MD USA (BPI). DNA extraction, polymerase chain reaction, and cycle sequencing of the nuclear ribosomal large subunit (LSU) were performed as previously described in Aime (2006). A DNA sequence of the LSU has been deposited in GenBank, accession number HQ412645.

Results

A 981 bp sequence of the 5'-end of the LSU was obtained. Blast analyses (Altschul et al. 1997) indicated placement within the *Pucciniaceae*, with closest similarities to other members of *Puccinia*, including *P. windsoriae* (98% similarity), *P. andropogonis* (98%), and *P. emaculata* (97%).

Taxonomy

Puccinia salihae Kirbağ & Aime **sp. nov.** Fig 1

Mycobank MB 519019

Aecia in fructus, folia, caulicola, 1 mm longa, 0.2 mm lata, meliaurantiata, cellulae peridii, sphaeroideae, elipsoideae 20 22.5 × 17.5 20 µm, parietibus 6 µm. Aeciosporae sphaeroideae, ovoidaeae, 20 22 × 18 20 µm, membrana 1 1.5 µm. Uredinia foliacolae, caulicolae, rarius fructicolae, 1 2 mm longa, 0.1 0.5 mm lata, pulvinata, flaventa. Urediniasporae globosae 30 35 × 25 27.5 µm, membrana 1.5 µm crassa, echinulataeae, poris germinationis 4 dispersis. Telia foliicolae, caulicolae, rarius fructicolae 1 2 mm longa, 0.1 0.5 lata, black, fuliginea, pulvinata, pulveraceae. Teliosporae elipticae-ovoideae 45 51 × 30 32 µm, membrane 1 1.5 µm, crassa in apice 4 µm, poro superiore apical, poro inferiore prope septum, crassa caducous stipite, hyaline, 10 25× 5 7.5 µm.

Type: Turkey, elaziğ-baskil, Hasan mountain, slope of Hacimustafa village, alt 1800 m on *Thymelaea aucheri* (*Thymelaeaceae*), 14 Jul 2007, leg: S. Kırbağ & M. Kursat (no:2000) (**Holotype** FUH 1272). Isotype in BPI (BPI 881123).

Etymology: Saliha, in honor of the mother of the first author.

Aecia (Fig 1c) on fruits, leaves and stems, 1 mm long, 0.2 mm wide, whitish to honey-orange in color, pinnately arranged. Peridial cells (Fig 1e) honey colored, irregularly shaped, in cross section spherical to ellipsoid, 20–22.5 × 17.5–20 µm, outer wall 1–1.5 µm thick, inner wall 4–5 µm thick. Aeciospores spherical to ovoid, 20–22 × 18–20 µm, spore wall 1–1.5 µm thick. Uredinia on leaves, stems, rarely on fruits, 1–2 mm long, 0.1–0.5 mm wide, yellowish, at first covered by epidermis, later rupturing. Urediniospores globoid 30–35 × 25–27.5 µm, wall 1.5 µm thick, echinulate, germ pores 4, scattered. Telia (Fig 1d) on leaves, stems rarely on fruits, 1–2 mm long, 0.1–05 mm wide, black, powdery. Teliospores (Fig 1f) ellipsoid-ovoid 45–51 × 30–32 µm, spore wall brown-chestnut, 1-1.5 µm thick, apex slightly thickened up to 4 µm, upper pore apical, lower pore near septum, pedicel hyaline, 10–25 × 5–7.5 µm.

Ecology, range & distribution: Known only from *Thymelaea aucheri* at the type location. Worldwide, the host plant has been recorded from Turkey, Syria, Lebanon, and western Iran (Davis 1985).

FIGURE 1. *Puccinia salihae* on *Thymelaea aucheri* (holotype): a–b. infected host plant; c. aecia; d. telia; e. peridial cells; f. teliospores.

Discussion

Thymelaeaceae is represented by three genera (*Diarthron*, *Daphne*, *Thymelaea*) in Turkey (Davis 1985). A single species of rust, *Uredo daphnicola* Dietel, has been reported on *Thymelaeaceae* from Turkey on *Daphne oleoides* Schreb. (Kabaktepe & Bahcecioglu 2005). *Uredo daphnicola* is considered

synonymous with *Melampsora daphnicola* (Dietel) Jørst. (Kuprevich & Transhel' 1957), a fungus that is readily distinguished from *Puccinia salihae* by its colorless urediniospores and paraphysate uredinia.

Worldwide, there are few rusts recorded from *Thymelaeaceae* and none from a species of *Thymelaea* (Farr & Rossman 2010). Only one other species of *Puccinia*, *P. gnidiae* Doidge on *Gnidia microcephala* Meisn. from South Africa, is known to infect a member of the family (Farr & Rossman 2010). In addition to host genus, *P. salihae* differs from *P. gnidiae* primarily by the much larger urediniospores (30–35 × 25–27.5 µm in *P. salihae* vs. 25–30 × 22.5–25 µm in *P. gnidiae*) and teliospores (45–51 × 30–32 µm in *P. salihae* vs. 30–40 × 25–30 µm in *P. gnidiae*) (Doidge 1939).

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Pucciniastrum enkianthi nom. nov., a replacement name for *P. hakkodaense*

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ABSTRACT *Pucciniastrum hakkodaense* (a rust pathogen of *Enkianthus*) is an illegitimate later homonym of *P. hakkodense* (a rust pathogen of *Leucothoe*). Hence, the replacement name *Pucciniastrum enkianthi* is proposed.

KEY WORDS *Naohidemyces*, nomen novum, nomenclature, *Thekopsora*

Pucciniastrum enkianthi Y.M. Liang & Kakish., **nom. nov.**

MYCOBANK MB519279

≡ *Pucciniastrum hakkodaense* Y.M. Liang & Kakish., Mycotaxon 92: 372. 2005, nom. illegit. (non *P. hakkodense* (S. Ito & Hirats. f.) Jørst. 1958).

ETYMOLOGY: referring to the host genus *Enkianthus*.

Pucciniastrum hakkodaense Y.M. Liang & Kakish. was described as a new species based on specimens on *Enkianthus campanulatus* (Miq.) G. Nicholson (*Ericaceae*) collected in Japan (Liang et al. 2005). However, following ICBN Art. 53.3 (McNeill et al. 2006), this name must be regarded as an illegitimate later homonym of *Pucciniastrum hakkodense* (S. Ito & Hirats. f.) Jørst. 1958 (≡ *Thekopsora hakkodensis* S. Ito & Hirats. f. 1927).

Thekopsora hakkodensis was described based on a specimen on *Leucothoe grayana* Maxim. (*Ericaceae*) from Japan (Hiratsuka 1927, 1936). Although Jørstad (1958) transferred the name to *Pucciniastrum*, Hiratsuka (1958) and Hiratsuka et al. (1992) continued to treat this species under its original name, *T. hakkodensis*. Subsequently, Sato et al. (1993) established a new genus *Naohidemyces* and treated *T. hakkodensis* as a synonym of the type species, "*Naohidemyces vaccinii*" S. Sato et al. (an invalidly published combination

based on the illegitimate teleomorph name *Melampsora vaccinii* G. Winter 1881, non (Alb. & Schwein.) G. Winter 1880). The correct name for this taxon is *Naohidemyces vacciniorum* (J. Schröt.) Spooner (Spooner & Butterfill 1999) based on the earliest legitimate teleomorph name, *Melampsora vacciniorum* J. Schröt. 1887.

Pucciniastrum enkianthi (≡ *P. hakkodaense*) is morphologically quite different from *Naohidemyces vacciniorum* (= *P. hakkodense*, *T. hakkodensis*). *Pucciniastrum enkianthi* produces its telia underneath the epidermis of the host whereas *N. vacciniorum* produces teliospores within epidermal cells of the host. In addition, their uredinial/telial hosts are not closely related though they are in the same family, *Ericaceae*: *P. enkianthi* on *Enkianthus*; and *N. vacciniorum* on *Hugeria*, *Leucothoe*, *Oxycoccus*, and *Vaccinium* (Sato et al. 1993, Liang et al. 2005).

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Morphological studies in *Dothideomycetes: Elsinoe* (*Elsinoaceae*), *Butleria,* and three excluded genera

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Abstract The types of the genera *Beelia*, *Butleria*, *Elsinoe*, *Hyalotheles*, and *Saccardinula* were examined to revise their familial position. The family *Elsinoaceae* (type: *Elsinoe canavaliae*) is described and its separation from *Myriangiaceae* is supported. *Butleria inaghatahani* has characters similar to *Elsinoaceae* where it should remain. *Beelia suttoniae* appears to be a superficial biotroph on the surface of leaves and thus *Beelia* should be placed in *Chaetothyriaceae* and is most similar to *Ainsworthia* (*Phaeosaccardinula*). Apart from the oblong to ovoid sessile asci in *Hyalotheles dimerosperma*, its placement in *Elsinoaceae* seems unwarranted, and *Hyalotheles* should be placed in *Dothideomycetes* incertae sedis. *Saccardinula guaranitica* may be better placed in *Microthyriaceae* or *Brefeldiellaceae*, because its ascomata greatly resemble thyrothecia found in *Microthyriaceae* and have similarities with *Brefeldiella*. Molecular sequence data from fresh collections is required to solve the problem of familial placement.

Key words — *Ascomycota*, morphology, taxonomy

Introduction

We are conducting studies on the *Dothideomycetes* in order to provide a natural classification (Zhang et al. 2008, 2009; Wu et al. 2010). As part of this research, we are restudying the type species of genera placed in the *Elsinoaceae*, a poorly known but relatively important family within the *Dothideomycetes* (Lumbsch & Huhndorf 2007). The *Elsinoaceae* presently comprise 10 genera including *Beelia*, *Butleria*, *Elsinoe*, *Hemimyriangium*, *Hyalotheles*, *Micularia*, *Molleriella*, *Saccardinula*, *Stephanotheca,* and *Xenodium* (Lumbsch & Huhndorf 2007). The family is characterized by immersed or erumpent ascomata,

composed of pale gelatinous thin-walled hyphal or pseudoparenchymatous cells. Asci are arranged in individual locules, in a single layer or are irregularly scattered, and are saccate to globose, bitunicate and fissitunicate. Ascospores are hyaline to brown, septate, or sometimes muriform (Kirk et al. 2001, 2008). The known anamorphs in *Elsinoaceae* are acervular coelomycetes with polyphialidic conidiogenous cells (e.g. *Sphaceloma* anamorphs of *Elsinoe*) (Sivanesan 1984). We have thus far examined several taxa within *Elsinoaceae* and in this paper report on the type species of *Elsinoe* and four poorly known genera: *Elsinoe canavaliae* (Raciborski 1900), *Beelia suttoniae* (Stevens 1925), *Butleria inaghatahani* (Saccardo 1914), *Hyalotheles dimerosperma* (Spegazzini 1908), and *Saccardinula guaranitica* (Spegazzini 1885). Full descriptions of these taxa and suggestion for their taxonomic placement are provided.

Materials & methods

Type specimens of *Elsinoe canavaliae*, *Beelia suttoniae*, *Butleria inaghatahani*, *Hyalotheles dimerosperma*, *Saccardinula guaranitica* were obtained from ZT & K, BISH, PAD, LPS and LPS, respectively. Ascomata were rehydrated in 3% KOH prior to examination and sectioning. Specimens were examined under a stereo microscope (Leica MZ16A) and fine forceps were used to remove one or two ascomata, which were mounted in water, Melzer's, Congo red, or cotton blue reagents. Observations and photographs were made under the light microscopes (Nikon E800 and Leica DM3000). Differential interference contrast microscopy was used for some hyaline structures.

Hand sections were cut with a sharp razor blade and thin (8 µm) sections were cut using a Leica CM1100 freezing microtome. The sections were transferred to a drop of water or a drop of cotton blue for examination and photography.

Taxonomy

Elsinoaceae Höhn. ex Sacc. & Trotter, Syll. fung. 22: 584 (1913).

Parasitic on leaves causing scabs and anthracnose. Ascomata immersed to erumpent, round or elongate, usually crustose, composed of pale gelatinous thin-walled hyphal or pseudoparenchymatous cells, opening by unordered breakdown of the surface layers. Specialized interascal tissue absent. Asci arranged in individual locules, in a single layer or irregularly arranged, saccate to globose, bitunicate, fissitunicate. Ascospores hyaline to brown, septate, sometimes muriform. Anamorph acervular where known.

Elsinoaceae was validated by Saccardo & Trotter (1913) based on the invalid non-Latin name "Elsinoëen" published by Höhnel (1909: 373). Höhnel (1909) regarded *Elsinoaceae* as a separate family at the same level with *Myriangiaceae* among "die Protodiscineen" or "die Plectascineen". Von Arx & Müller (1975) extended the family concept and placed *Elsinoe* with another 15 genera in *Myriangiaceae*. Luttrell (1973) used this concept to unite seven additional genera with *Elsinoe* in *Myriangiaceae*. Barr (1979) and Eriksson (1981) maintained

the *Elsinoaceae* as separate from *Myriangiaceae*, mainly based on habit and developmental studies. Lumbsch & Huhndorf (2007) placed *Elsinoaceae* in the order *Myriangiales* and this is supported in the studies of Schoch et al. (2006, 2009) and Boehm et al. (2009). Further molecular study is needed to establish whether *Elsinoaceae* and *Myriangiaceae* are distinct families, although the evidence points towards this (Schoch et al. 2009).

TYPE GENUS: ***Elsinoe*** Racib., Paras. Alg. Pilz. Java's (Jakarta) 1: 14 (1900).

Ascomata parasitic, usually forming scabs or anthracnose on leaves. Ascomata pulvinate, white or occasionally brown, in section with numerous locules distributed inside the ascostromata, with numerous asci inside each locule. Paraphyses absent. Asci 8-spored, bitunicate, globose to subglobose, without a pedicel. Ascospores hyaline, transseptate or muriform.

ANAMORPH: *Sphaceloma* spp. Acervuli pseudoparenchymatous from which hyaline to pale-brown phialidic conidiophores and/or conidiogenous cells originate. Conidia hyaline, unicellular, ellipsoidal, biguttulate.

TYPE SPECIES: ***Elsinoe canavaliae*** Racib., Paras. Alg. Pilze Java's 1: 14 (1900), as "*cavavalliae*". FIG. 1

Ascomata parasitic on leaves, forming scabs on lower surface (FIG. 1A). Ascomata pulvinate, white or occasionally brown, 1.8–3.3 mm in diam., gregarious, immersed or erumpent, irregularly shaped, spreading around the host veins (FIG. 1B–C), in section with numerous locules distributed inside the ascostromata, with numerous asci inside each locule, with lower part of ascomata fusing with the hyaline host cells (FIG. 1D–E). Paraphyses not seen, probably absent. Asci 19–23 × 16–20 µm (mean = 21 × 18 µm, n = 10), 8-spored, bitunicate, globose to subglobose, without a pedicel, with a wide but indistinct ocular chamber (FIG. 1F–H). Ascospores 14–18 × 6–9 µm (mean = 15 × 7 µm, n = 10), irregularly arranged, hyaline, ellipsoidal-fusiform, 3–4 septate, constricted at the central septum, upper part wider with slightly acute ends, lower part narrow with rounded ends, guttulate (FIG. 1I–J).

ANAMORPH: *Sphaceloma*.

SPECIMEN EXAMINED: INDONESIA, JAVA, on leaves of *Canavalia gladiata* (Savi) DC. (*Fabaceae*) leg. Raciborski (ZT Myc 1489, lectotype; K 164015, syntype).

Elsinoe was established by Raciborski (1900) with descriptions of three species (*E. canavaliae, E. antidesmae* Racib., *E. menispermacearum* Racib.). Von Arx & Müller (1975) placed *Elsinoe* in *Myriangiaceae* based on its immersed or erumpent, pulvinate or irregular ascomata and being parasitic on the leaves of higher plants causing scabs. Later, the genus was removed to the family *Elsinoaceae* (Barr 1979, Lumbsch & Huhndorf 2007). The more than 141 species recorded for *Elsinoe* (www.indexfungorum.org 2010) are generally parasites on leaves, stems, scale insects or other fungi.

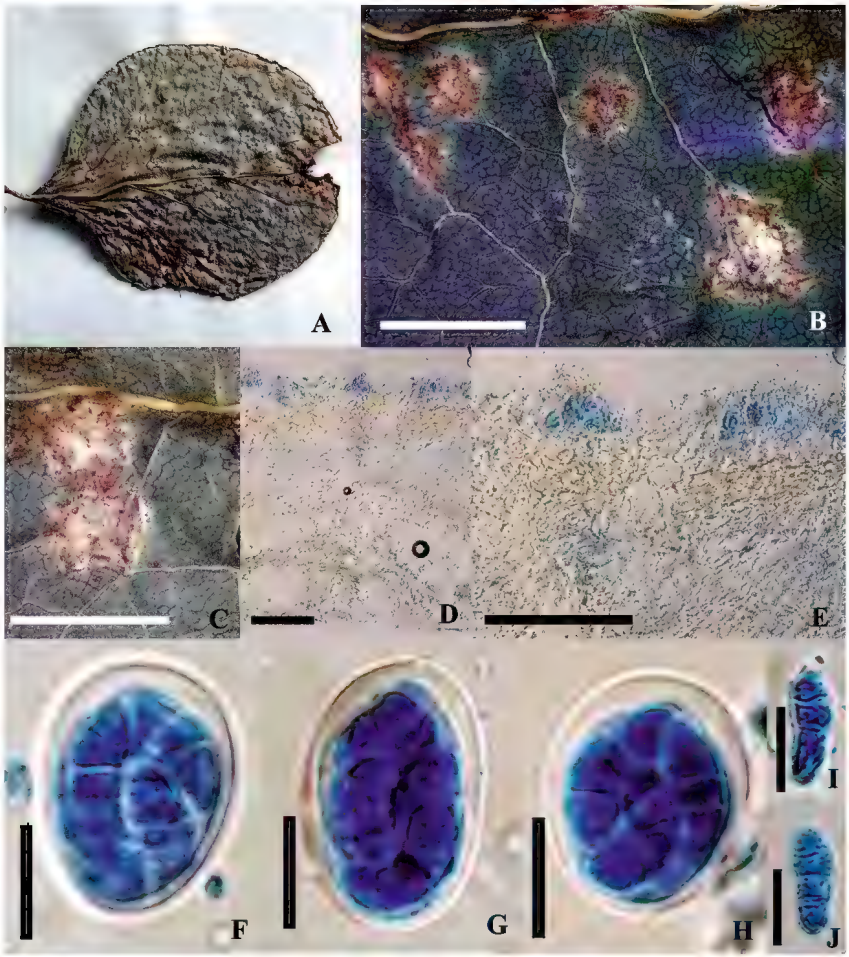


FIG. 1. *Elsinoe canavaliae* (lectotype) A. Appearance of ascomata on the host surface of leaves. B–C. Appearance of ascomata on the leaves showing the irregular shape of scabs. D–E. Vertical section through ascomata in cotton blue showing the ascomata with numerous asci inside each locule. F–H. Sessile asci in cotton blue. I–J. Ascospores in cotton blue.
Scale bars: B– C 5 mm, D– E 100 µm, F–J 10 µm.

This is an important plant pathogenic genus causing scab and anthracnose of *Citrus, Malus, Rubus, Vitis* spp. and other hosts and descriptions and disease symptoms can be found on the worldwide web. It is important however, to characterize the type of the genus, which is less well known. *Elsinoe fawcettii* Bitanc. & Jenkins and *E. australis* Bitanc. & Jenkins cause scab diseases of *Citrus* species (Hanlin 1989; Timmer et al. 1996), and there are many other important pathogens in this genus. Examples include *Elsinoe veneta* (Burkh.)

Jenkins, which causes cane spot of raspberry (Munro 1988); *E. dracophylli* P.R. Johnston & Beever, which causes spots on *Dracophyllum* (Johnson and Beever 1994); and *E. mangiferae* Bitanc. & Jenkins (Bitancourt & Jenkins 1946), which causes mango scab. *Elsinoe takoropuku* G.S. Ridl. & Ramsfield is a recently introduced species (Ridley & Ramsfield 2006), but it differs quite markedly from *E. canavaliae* as it does not cause scabs on leaves, but forms ascostromata on twigs with locules each containing single asci. The asci are thought to be more like the *Elsinoe* type, although the fungus shares many characters with *Myriangiaceae*, illustrating a clear need for molecular studies on this group.

There have been a few molecular studies incorporating strains of *Elsinoe*. Schoch et al. (2006, 2009) and Boehm et al. (2009) showed the species to cluster in the *Myriangiales* and form a distinct subclade – the *Elsinoaceae*. However, since only four *Elsinoe* specimens and one *Myriangium* specimen were used in Schoch et al. (2006) and no *Elsinoe* specimens were used in Schoch et al. (2009), the molecular data does not conclusively resolve two separate families (*Elsinoaceae* and *Myriangiaceae*). Swart et al. (2001) analyzed ITS sequence data of six *Elsinoe* species — *E. banksiae*, *E. leucospermi*, *E. proteae*, *Elsinoe* sp. (from *Citrus*), *Elsinoe* sp. (from *Banksia*), *Sphaceloma protearum* — in their research on the taxonomy of species associated with scab disease of *Proteaceae;* their molecular analyses supported five species, of which four were described in that paper. A molecular study of many more *Elsinoe* species is needed.

Beelia F. Stevens & R.W. Ryan, in Stevens, Bulletin of the Bernice P. Bishop Museum 19: 71 (1925).

Forming colonies on the surface of the leaves. Ascomata black, superficial, ostiolate, aparaphysate, borne on free, brown, septate, branching mycelium. Asci bitunicate, broadly ellipsoidal, obovate to saccate, pedicellate. Ascospores cylindrical, hyaline to straw-coloured, 6-celled.

Anamorphs unknown.

Type species: ***Beelia suttoniae*** F. Stevens & R.W. Ryan, in Stevens, Bulletin of the Bernice P. Bishop Museum 19: 71 (1925). Fig. 2

Ascomata on the upper surface of leaves, scattered beneath and between darkened mycelial strands (Fig. 2A–B). Ascomata 190–210 µm wide × 115–133 µm high, superficial, globose to subglobose, black, with a flattened base which is easily removed from the substrate containing numerous asci (Fig. 2C–D). Peridium 25–30 µm wide, up to 39 µm wide at the apex, 20 µm wide at the base; comprising two cell types; outer cells brown, thick-walled, textura globulosa, inner cells thin-walled, lighter, textura angularis (Fig. 2D–E). Paraphyses not seen, probably absent. Asci 70–89 × 45–55 µm (mean = 84.3 × 51.2 µm, n = 20), 8-spored, bitunicate, broadly ellipsoidal, obovate to saccate, thick-walled, with small pointed pedicle, and with ocular chamber up to 24.4 µm wide × 14.9 µm

FIG. 2. *Beelia suttoniae* (syntype): A. Appearance of ascomata on host leaf scattered beneath and between darkened mycelium. B. Squash of ascoma in water. C–E. Vertical section through ascoma showing outer and inner cell types. F–G. Asci. Note the bitunicate appearance and ocular structure in the extended apex. H–I. Hyaline ascospores with 5 septa. Note the central septum is strongly constricted and upper part wider.

Scale bars: A, B 100 µm. C–E 50 µm, F 25 µm. G 50 µm. H–I 10 µm.

high (FIG. 2F–G). Ascospores 38–45 × 13–18 µm (mean = 42.8 × 14.6 µm, n = 20), irregularly arranged, cylindrical, hyaline, 5-septate, strongly constricted at each septum, central septum very strongly constricted and upper part wider, smooth-walled, with narrow mucilage sheath (FIG. 2 H–I).

ANAMORPH unknown.

Specimen examined: USA, Hawaii, on leaves of *Suttonia lanaiensis* Mez (*Myrsinaceae*), circa 1925, Lanai, no. 421, leg. Munro (BISH 499845, syntype).

Beelia was introduced by Stevens & Ryan (Stevens 1925) and remained monotypic until *B. philippinensis* Bat. & C.A.A (Batista & Costa 1959) and *B. plumeria* Bat. & Cavalc. (Batista et al. 1967) were added. Stevens (1925) placed *Beelia* in the family *Microthyriaceae,* where it was accepted by Petrak (1953). Von Arx & Müller (1975) transferred *Beelia* to the *Myriangiaceae* based on its dimidiate ascomata and long (> 30 µm) brown ascospores. Hawksworth et al. (1995) later transferred the genus to the *Elsinoaceae,* a placement accepted by Kirk et al. (2001) and Lumbsch & Huhndorf (2007). The taxon appears to be a superficial biotroph on leaf surfaces, a character shared by genera in *Chaetothyriaceae.* As *Beelia* seems most similar to *Ainsworthia* (Batista & Ciferri 1962; = *Phaeosaccardinula* Henn., fide von Arx & Müller 1975) in that family, we suggest that the genus may belong in *Chaetothyriaceae.* New collections and molecular analyses are needed to clarify the familial placement.

Butleria Sacc., Annls mycol. 12(3): 302 (1914).

Forming leaf spots. Ascomata gregarious, superficial, black, subglobose, with numerous locules distributed at different levels inside the ascomata, with only one globose to oblong asci inside each locule. Asci 8-spored, bitunicate, sessile. Ascospores oblong to ovoid, brown, two-celled.

Anamorphs unknown.

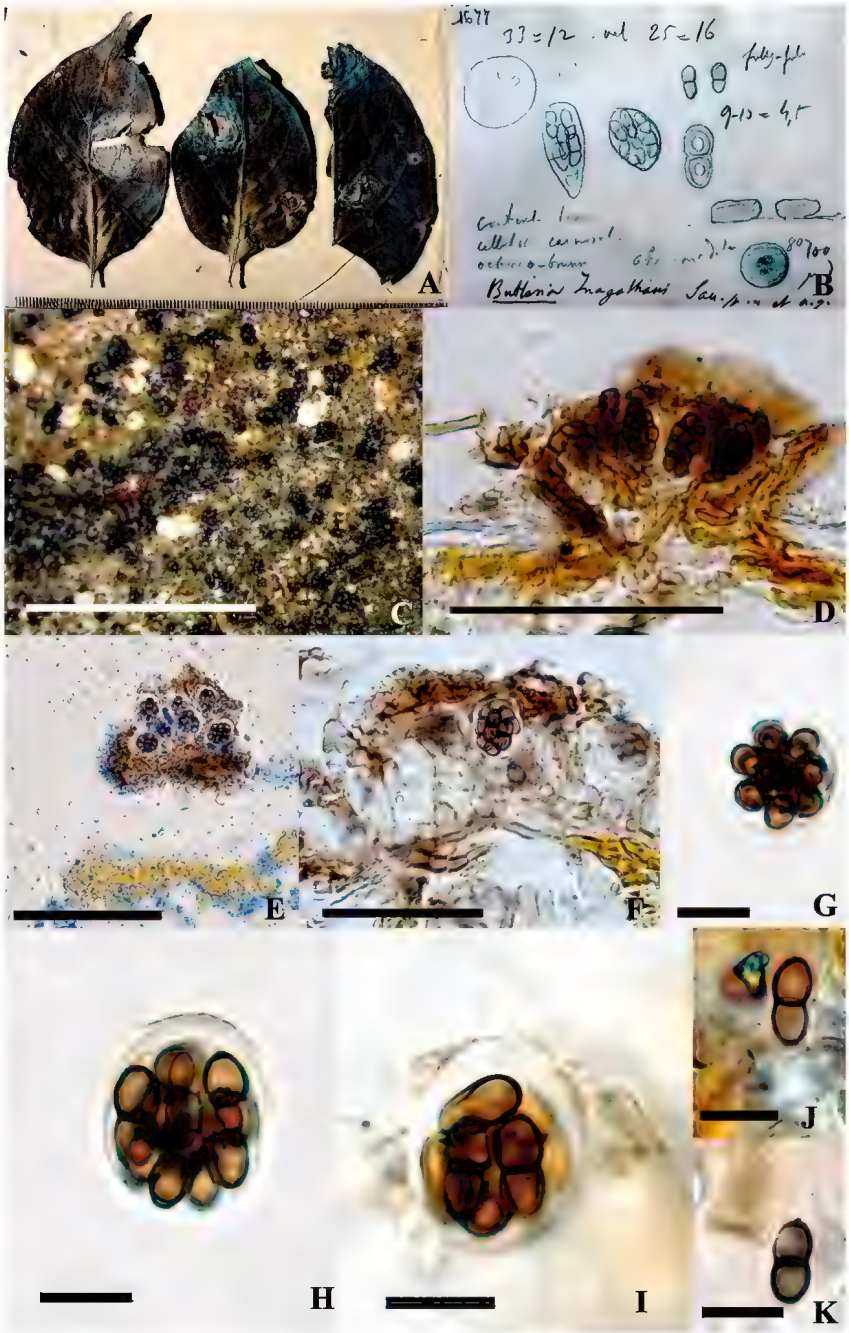
Type species: ***Butleria inaghatahani*** Sacc., Annls mycol. 12(3): 302 (1914). Fig. 3

Forming light, somewhat zonate target spots on leaves (Fig. 3A,C) with minutely stromatic ascomata forming on the upper surface. Ascomata 81–130 × 60–88 µm (mean = 114.4 × 75.2 µm, n = 15), gregarious, superficial, ovoid, subglobose to globose, black, with numerous locules distributed at different levels inside the ascomata, with only one globose to oblong asci inside each locule (Fig. 3B, D–F). Paraphyses not seen. Asci 19–25 × 16–23 µm (mean = 22.7 × 19.2 µm, n = 15), 8-spored, bitunicate, globose to oblong, sessile, with small ocular chamber up to 11.8 µm wide × 2.9 µm high (Fig. 3G–I). Ascospores 8–13 × 4–6 µm (mean = 12.1 × 4.8 µm, n = 15), irregularly arranged in rows of 3 or 4, oblong to ovoid, brown, 2-celled, constricted at the central septum, upper cell slightly larger than lower cell, spinulose (Fig. 3J–K).

Anamorph: unknown.

Specimen examined: BANGLADESH, Comillae District, Krishnapone, associated with target spots on leaves of *Vangueria* sp. (*Rubiaceae*), causing, 8 December 1913, leg. Inaghataban (PAD 1677, holotype).

Butleria is a monotypic genus established by Saccardo (1914) for *B. inaghatahani.* Von Arx & Müller (1975) referred this genus to *Myriangiaceae* based on its small bright ascomata and 2-celled ascospores. Currently, *Butleria* is placed in the family *Elsinoaceae* (Barr 1979; Lumbsch & Huhndorf 2007; Kirk et al. 2001,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2008). This taxon does appear to be a candidate for *Elsinoaceae* as it is a parasite on leaves and has single asci locules scattered throughout a somewhat reduced but pulvinate ascomata. The ascospores are, however, distinct in being brown. Here also additional collections and molecular analyses are needed to clarify family relationships.

Hyalotheles Speg., Revta Mus. La Plata 15(2): 11 (1908).

Forming black spots on the upper surface of leaves. Individual ascomata scattered, superficial, globose, black. Paraphyses not seen. Asci 8-spored, bitunicate, oblong to obovoid, without a pedicel. Ascospores irregularly arranged in 2---3 rows, globose, hyaline, 1-celled, minutely guttulate.

ANAMORPH: unknown.

TYPE SPECIES: ***Hyalotheles dimerosperma*** Speg., Revta Mus. La Plata 15(2): 11 (1908). FIG. 4

Forming black spots on the upper surface of leaves (FIG. 4A,C). Individual ascomata scattered, superficial, globose, black, composed of the hyaline pseudoparenchymatous cells at the edge and brown to dark pseudoparenchymatous cells at the center (FIG. 4B,D–E). Ascomata in section 50–62 × 54–60 µm (mean = 56 × 55 µm, n = 10) (FIG. 4E–F). Peridium dark to brown, up to 4–5 µm wide (FIG. 4E-F). Paraphyses not seen. Asci 23–28 × 15–18 µm (mean = 26 × 16 µm, n = 10), 8-spored, bitunicate, oblong to obovoid, without a pedicel (FIG. 4G–H). Ascospores 5–7 × 5–7 µm (mean = 5.5 × 6. µm, n = 8), irregularly arranged in 2–3 rows, globose, hyaline, 1-celled, minutely guttulate (FIG. 4I).

ANAMORPH: unknown.

SPECIMEN EXAMINED: BRAZIL, SAO PAULO, Casa do Isolamento, on leaves of *Rubus urticifolius* Poir. (*Rosaceae*), collector unknown (LPS 408, holotype).

Hyalotheles is a monotypic genus introduced by Spegazzini (1908) for *H. dimerosperma*. The genus is characterized by ascomata developing on glandular hairs of the host and spherical ascospores (von Arx & Müller 1975). Von Arx & Müller (1975) placed *Hyalotheles* in the family *Myriangiaceae,* but later Barr (1979) transferred the genus to the family "*Saccardinulaceae*" [an invalid name]. Lumbsch & Huhndorf (2007) now recognize *Hyalotheles* in the family *Elsinoaceae*. Apart from the oblong to ovoid sessile asci, however, placement in *Elsinoaceae* seems unwarranted, and *Hyalotheles* is better referred to *Dothideomycetes* incertae sedis. Additional collections and molecular analyses are needed to clarify family relationships.

FIG. 3 (left). *Butleria inaghatahani* (holotype): A, C. Appearance of ascomata in leaf spot. B. Drawing from herbarium specimen. D, F. Vertical section through ascomata showing the part connected to the leaf. E. Vertical section through ascomata (in cotton blue reagent). G I. Saccate asci. J K. Ascospores. Scale bars: C 1 mm, D E 100 µm, F 50 µm, G K 10 µm.

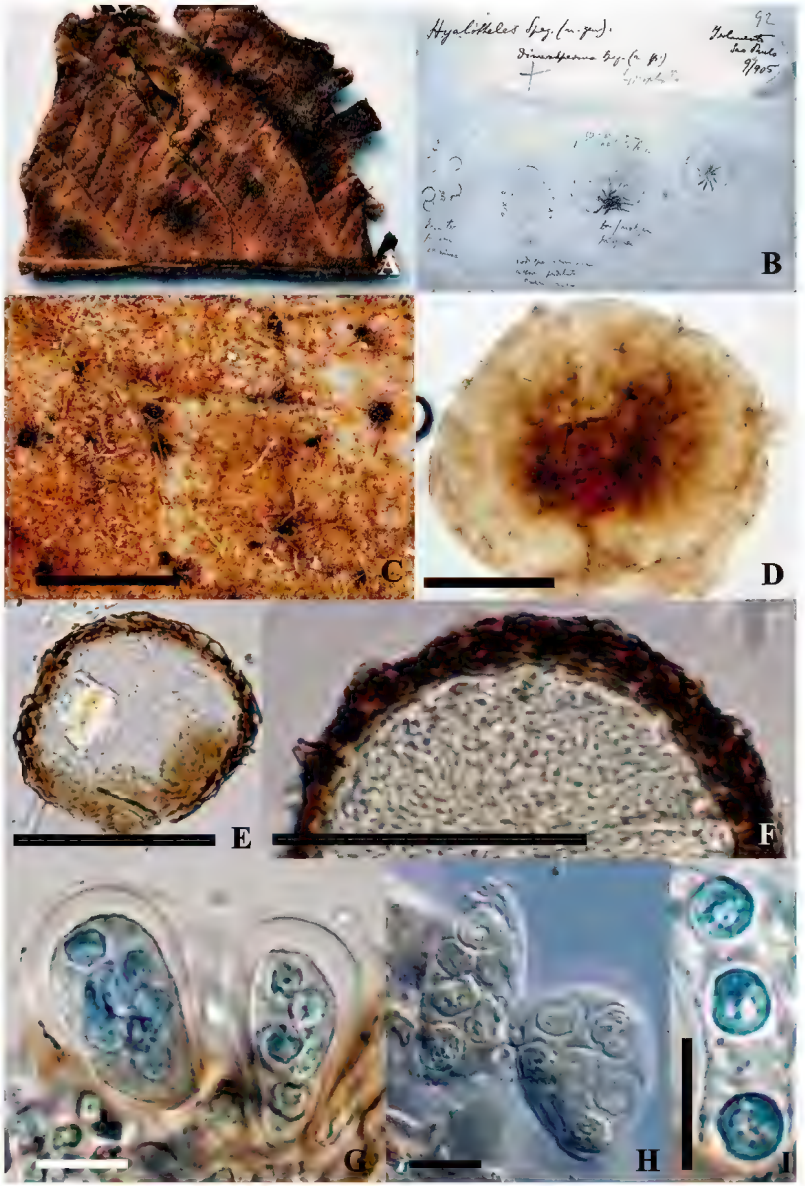


FIG. 4. *Hyalotheles dimerosperma* (holotype). A, C. Appearance of ascomata on host surface of leaf. B. Appearance of the drawing picture from the specimen. D. Ascomata in lactic acid showing the structure of dark to brown pseudoparenchymatous cells at the center and hyaline pseudoparenchymatous cells at the edge. E–F. Vertical section through ascoma in cotton blue. G–H. Asci in cotton blue. I. Ascospores in cotton blue.
Scale bars: C 1 mm, D 100 µm, E 50 µm, F 100 µm, G–H 10 µm.

Saccardinula Speg., Anal. Soc. cient. argent. 19(6): 257 (1885).

Thalli forming raised black spots on the lower leaf surface. Individual ascomata gregarious, superficial, very easily removed from host surface, ovoid to globose, black, with the two different regions; the central region comprising dark brown radiating cells with the ascomata and the marginal parts comprising lighter brown radiating cells. Ascomata subglobose, fusing with one or more ascomata, with a single locule distributed inside each ascoma, with single asci inside each locule. Paraphyses not seen. Asci 8-spored, bitunicate, globose to oblong, without pedicel. Ascospores ellipsoid-fusiform, with a few transsepta and occasional longitudinal septa.

ANAMORPH: unknown.

TYPE SPECIES: ***Saccardinula guaranitica*** Speg., Anal. Soc. cient. argent. 19(6): 258 (1885). FIG. 5

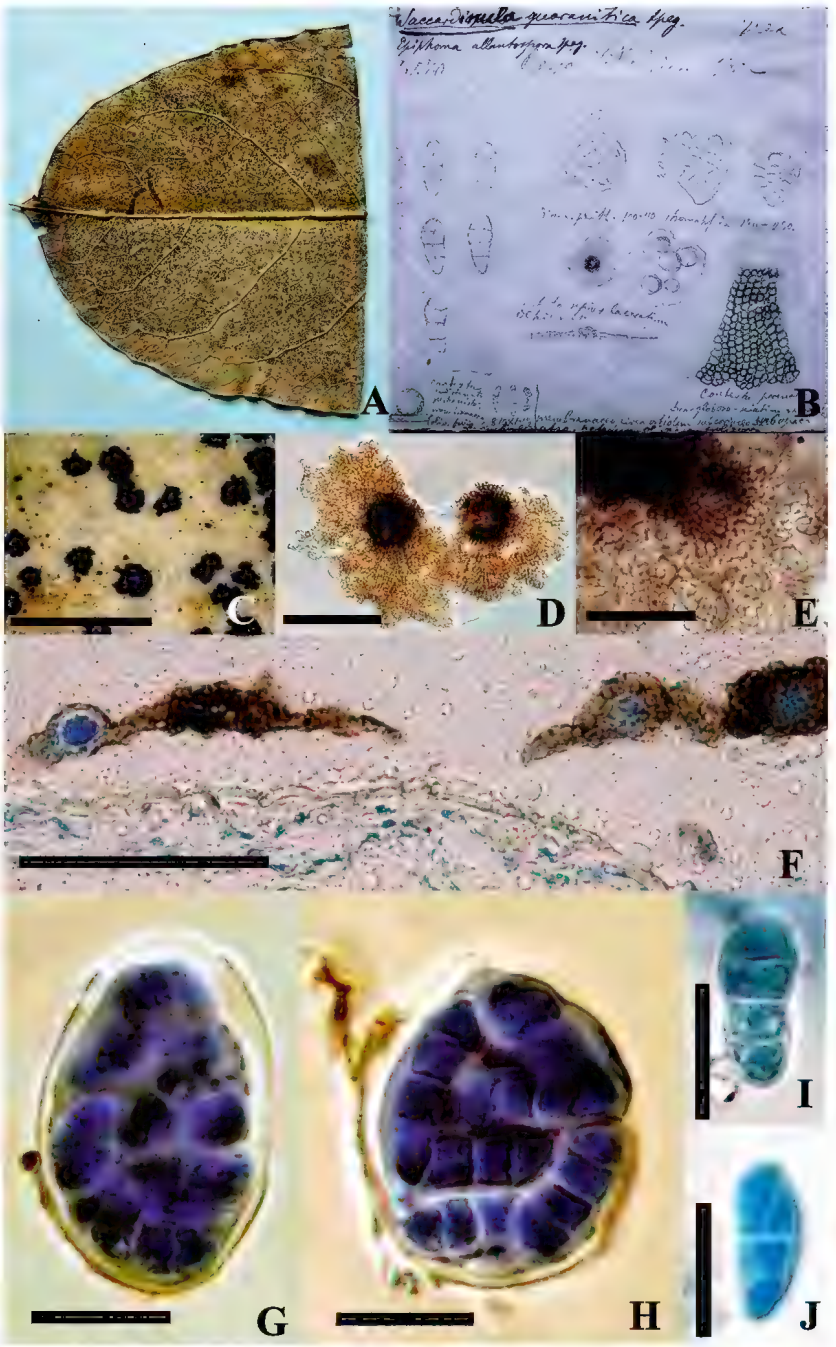
Thalli forming raised 400 µm black spots on the lower leaf surface (FIG. 5A–C). Individual thalli gregarious, superficial, very easily removed from host surface, ovoid to globose, black, with the two different regions; the central region comprising dark brown radiating cells with the ascomata and the marginal parts comprising lighter brown radiating cells to 3–7 µm in diam. (FIG. 5B,D–E). Ascomata in section 81–130 µm × 60–88 µm, subglobose, fusing with one or more ascomata, with a single locule distributed inside each ascoma, with single asci inside each locule (FIG. 5F). Paraphyses not seen. Asci 19–25 × 16–23 µm (mean = 22 × 20 µm, n = 10), 8-spored, bitunicate, globose to oblong, without pedicel (FIG. 5G–H). Ascospores 8–13 × 4–6 µm (mean = 10 × 5 µm, n = 10), irregularly arranged, ellipsoid-fusiform, 2–3 septate, constricted at the central septum, one end broadly rounded and slightly pointed at the other end, hyaline, occasionally with longitudinal septa, guttulate (FIG. 5I–J).

ANAMORPH: unknown.

SPECIMEN EXAMINED: BRAZIL, Borga, Villa Rice, on leaves of *Ilex sp.*, (*Aquifoliaceae*), January1882, leg. B. Balanse (LPS 1469, holotype).

Saccardinula was erected by Spegazzini (1885) for *S. guaranitica*. Luttrell (1973) placed this genus in the "*Saccardinulaceae*" based on its ascostromata grouped in a radiate, superficial, cellular membrane. Von Arx & Müller (1975) removed this genus to *Myriangiaceae* based on its small ascomata, which are pustulate at the centre, and short (≤ 20 µm) hyaline ascospores, but Barr (1979) retained the genus in the "*Saccardinulaceae*." Lumbsch & Huhndorf (2007) placed *Saccardinula* in the family *Elsinoaceae*.

Re-examination of *S. guaranitica* shows that the ascomata greatly resemble the thyrothecia found in *Microthyriaceae* (Wu et al. 2010), although *Brefeldiella* Speg. (*Brefeldiellaceae*), which occurs on leaves and has ascomata comprising a wide area of radiating cells and a darker central raised area in which the asci form, may be more similar (Reynolds & Gilbert 2005). *Elsinoaceae* is characterized by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round or elongate ascomata composed of pale gelatinous thin-walled hyphal or pseudoparenchymatous cells and which open by unordered breakdown of the surface layers (Kirk et al. 2001). Therefore, *Saccardinula* might better be placed in either *Microthyriaceae* or *Brefeldiellaceae*, or "*Saccardinulaceae*" could be validated to accommodate it (Eriksson 1981). Ultimately only molecular sequence data will solve the problem of familial placement.

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FIG. 5 (left). *Saccardinula guaranitica* (holotype) A, C. Appearance of ascomata on the host surface of leaf. B. Drawing from herbarium specimen. D E. Ascomata in lactic acid showing the two different regions; the central region comprising dark brown radiating cells and the marginal parts comprising lighter brown radiating cells. F. Vertical section through ascoma. G H. Asci in cotton blue reagent. I J. Ascospores in cotton blue reagent.

Scale bars: C 1mm, D 100 μm, E 50 μm, F 100 μm, G J 10 μm.

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Book reviews and notices

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Introduction

Lichens, the genus *Amanita*, stereoid fungi, rust fungi, and a checklist for the fungi in Japan are the focus for the book reviews presented here, just an average anthology of today's systematic literature. The need to inventory, classify and describe is as great as ever, or even more pressing in light of the changes in the environment that take place, and the books reviewed here fill, in their own small ways, the existing knowledge hole.

This contribution concludes with a list of newly published books to be included in upcoming Book reviews and notices.

Ascomycetes

***Hypotrachyna* (*Parmeliaceae*, lichenized fungi).** By H.J. Sipman, J.A. Elix & T.H Nash III. 2009. New York Botanical Garden Press, Bronx, New York, NY 10458-5126, USA; <www.nybgpress.org>. Pp. 179, figs 83, tables 2. [Flora Neotropica Monograph 104] ISBN 978-0-89327-502-0. Price: US $ 48.

Works such as this form the backbone of biodiversity inventory and conservation work. They are of particular importance where they are the culmination of extensive study and field experience and deal with groups for which there was previously no contemporary synthetic works in the region concerned. The tropical American species of this genus of parmelioid lichens had previously been studied by Hale (1975), who accepted 74 species. However, from the work of Sipman and a growing band of lichenologists in the region, it had become clear that there were numerous additional species to be

* Books for consideration for coverage in this column should be mailed to the Book Review Editor at the address above. All unsigned entries are by the Book Review Editor.

accommodated. This new monograph almost doubles the number of known species to 140, of which 37 are new to science, at the same time relegating many names to synonymy. *Hypotrachyna* is primarily a tropical genus, although a few species are known in temperate regions where they tend to occur in somewhat high-rainfall regions. The neotropics in particular are their centre of diversity, and 85 of the species treated here are only known from that region.

The monograph follows the traditional pattern of an historical overview, a discussion of morphological and anatomical characters, a rather full account of the 109 lichen products detected, notes on ecology and biogeography, and a key. The species are arranged alphabetically with full bibliographical and type citations (almost all types having been studied by at least one of the authors) for accepted names and synonyms, descriptions, references to illustrations, details of chemical characters, a summary of the distribution, details of specimens or selected specimens examined, and notes concentrating on separation from other species. Distribution maps are provided in many cases, but the halftone photographs are mainly of the types of newly described species or lectotypes. Photographs of more species, especially if they could have been in colour, would have been a valuable addition to facilitate the use of the monograph by non-specialists who lack immediate access to the works containing the listed illustrations. I was pleased to see that the Latin diagnoses were generally restricted to 2–3 lines but sad that data on the new names had not been deposited in MycoBank, and consequently reference numbers not included. For me, and I suspect many potential users, the key, in addition to being presented in the space-wasting indented and dated format used in other volumes of the series, places too much emphasis on chemical characters. Is it reasonable to expect users to have a UV-lamp and potassium hydroxide to hand to get beyond the second couplet? There is still perhaps too much emphasis on chemical products in the formulation of species concepts here, and I was disappointed to see the "fertile species"/"asexual counterparts" concept perpetuated — in all such supposed cases in macrolichens so far studied in detail by molecular methods the relationships have proved to be unsound (Crespo & Pérez-Ortega 2009). Following Hale, conidial characters are not considered taxonomically valuable and seem not to have been studied in most species, but I was pleased to see ascospore size differences featured in a few of the species separations — as with conidia, ascospore characters are much-neglected in macrolichen systematics.

No molecular studies were undertaken in the course of this revision, and it would have been of interest to know to what extent the more widely distributed species, such as *Hypotrachyna revoluta*, are really intercontinental as opposed to further instances of cryptic speciation as is proven frequent in other "widespread" parmelioid lichens (Crespo & Lumbsch 2010). I suspect

modifications of species concepts may become necessary in the future in some cases, but that will be a task for the next generation of researchers who can proceed from the base now created. Overall, however, this is a scholarly landmark work on the genus, and one that has been painstakingly compiled over many years by three of today's most experienced lichenologists. It can be unhesitatingly commended as an authoritative account of the current state of knowledge of this important lichenized genus.

Crespo A, Lumbsch HT. 2010. Cryptic species in lichen-forming fungi. IMA Fungus 1: 167–170.

Crespo A, Pérez-Ortega S. 2009. Cryptic species and species pairs in lichens: a discussion on the relationship between molecular phylogenies and morphological characters. Anales del Jardin Botánico de Madrid 66 (S1): 71–81.

Hale ME. 1975. A revision of the lichen genus *Hypotrachyna* (*Parmeliaceae*) in tropical America. Smithsonian Contributions to Botany 25: 1–73.

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The lichen genus *Rinodina* (Ach.) Gray (*Lecanoromycetidae, Physciaceae*) in North America, north of Mexico. By J.W. Sheard. 2010. National Research Council of Canada, Ottawa, Ontario ON K1A 0R6, Canada <pubs@nrcresearchpress.com>. Pp. viii + 246, figs 182, tables 6. [Monograph Publishing Program.] ISBN 978-0-660-19941-2. Price: US $ 89.95.

John Sheard has made the genus *Rinodina* the primary focus of his systematic research since he completed his PhD on the British and European species in 1966; after he moved to the University of Saskatchewan in 1968 his attention was immediately drawn to the North American species — on which he proceeded to make contributions from time to time while accumulating information for this work. Early retirement and appointment as an Emeritus Professor of the University in 2000 has enabled him to bring this major work to fruition. Since the work of Malme in 1902, ascospore structural differences have become the fundamental criterion in the classification of the genus. The two developmental ascospore types (*Dirinaria*-type, *Physcia*-type) yield no fewer than 13(-14) spore types amongst the North American species. Various molecular studies during the last decade have shown that the genus is paraphyletic (e.g. Kaschik 2006) with foliose genera of the *Physciaceae* (e.g. *Anaptychia*, *Physcia*, *Physconia*) nested within it. While not addressing this issue, Sheard does reflect on the ancient origins of different groups within the genus in relation to continental movements, suggesting that a calcicolous group well represented in Colorado could date back to the Middle Triassic (240-230 Myr). Several other

biogeographical hypotheses are also put forward for future testing, among them molecular, and there are detailed discussions of regional diversity and "floristic" elements that can be recognized within North America.

The introductory chapters include information on the 61 characters assessed for each specimen studied in detail and recorded in a spreadsheet — a method rarely used these days but routine during numerical (phenetic) analyses in the 1960s and 1970s. Especially important is the discussion of spore types, and the illustrations make clear how important it is to observe developing as well as mature (but not over-mature) ascospores when considering to which category they should be assigned; here clearing in Melzer's reagent is recommended for material less than 2-3 years old. The numerous half-tone photographs in this section and in the species accounts make these characters much clearer than line drawings alone, as used in some other regional accounts of the genus. The chemical products detected are summarized, but these are much less diverse than in many other lichenized genera. The key is strictly dichotomous, but in three categories arranged by substratum. In general this will work well, but as Sheard notes substrate-switches can occur so some caution in their use would be prudent. Spore types unavoidably feature strongly in the keys. Otherwise the characters seem clear, although I would have preferred to see maximal and minimal spore lengths rather than averages used in several couplets.

Species treatments are particularly detailed, especially with respect to descriptions of anatomical features, and there are the expected notes on ecology, distribution, and characterization. Lists of specimens examined and also in many cases distribution maps are provided, the latter with records split into pre- and post-1960 categories. In all, 96 species are recognized in North America, of which five are newly described and ten had not previously been reported. Of these, 36 are evidently endemic. At the same time, no fewer than 30 species, including 13 first described from North America, are placed into synonymy. In all cases detailed bibliographic and typification details are presented. Notes are provided on some excluded species, including the lichenicolous "*Rinodina*" *insularis,* which has *Bacidia*-type (not *Lecanora*-type) asci and which Sheard considers to be best treated as "an aberrant *Buellia*" for the present. In fact, this species has now been included in *Endohyalina* by Giralt et al. (2010), a work which evidently came out while Sheard's monograph was in press.

This meticulously produced monograph is a great tribute to Sheard's almost lifelong dedication to the genus and to his powers of critical observation. It should be looked at by others contemplating monographs of crustose lichens as a standard to aspire to. This revision is sure to remain the pivotal work on species concepts in the genus for many years to come, even if the genus itself is eventually divided as a consequence of more intensive molecular work and a re-examination of generic concepts in the family. The work also has value as a

complement to the several regional revisions of the genus produced in recent decades (e.g. Mayrhofer & Poelt 1979, Mayrhofer 1984, Giralt 2001, Mayrhofer & Moberg 2002, Kaschik 2006) and so merits use worldwide to. This is a job extremely well done in the best taxonomic tradition!

Giralt M. 2001. The lichen genera *Rinodina* and *Rinodinella* (lichenized ascomycetes, *Physciaceae*) in the Iberian Peninsula. Bibliotheca Lichenologica 79: 1–160.

Giralt M, van den Boom PPG, Elix JA. 2010. *Endohyalina*, the genus in the *Physciaceae* to accommodate the species of the *Rinodina ericina*-group. Mycological Progress 9: 37–48.

Kaschik M. 2006. Taxonomic studies on saxicolous species of the genus *Rinodina* (lichenized ascomycetes, *Physciaceae*) in the Southern Hemisphere with emphasis in Australia and New Zealand. Bibliotheca Lichenologica 93: 1–162.

Mayrhofer H. 1984. Die saxicolen Arten der Flechtengattung *Rinodina* und *Rinodinella* in der alten Welt. Journal of the Hattori Botanical Laboratory 55: 327-493.

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Basidiomycetes

Quelques espèces nouvelles ou mal délimitées d'*Amanita* de la sous-section *Vaginatinae*. 1° complément à *Amaniteae*, Fungi Europaei 9. By P. Neville † & S. Poumarat. 2009. Fungi non delineati LI-LII. Edizioni Candusso, Via Ottone Primo 90, 17021 Alassio SV, Italy. <maxcandusso@libero.it>. Price € 26.00.

During the final illness of the late Professor Pierre Neville and after his death, several friends and associates generously expended time and effort in the production of this book. It represents their personal tribute to him, his life, and his work. It also comprises the extant fragments of the work of Neville and Poumarat on *Amanita* subsection *Vaginatinae*, which I understand to be section *Vaginatae* in the sense of Corner and Bas (1962) as revised by Yang (1997). A publication covering European taxa of *Amanita* [section *Amanita*] subsection *Amanitella* and subsection *Vaginatinae* was proposed to follow the extensive volume on the *Amanitaceae* [p.p.] of Europe written by Neville and Poumarat (2004; reviewed in Mycotaxon 92: 474-484). Understandably, the present volume has a much more restricted scope. It is significant that "Europe" is not mentioned in the title of the present work. The materials on which the reported results are based are almost exclusively from France, Italy, and Spain. I found one collection each from Finland and Switzerland listed as revised material.

For a book discussing taxa that first appeared in the works of Schaeffer and Secretan, it is a shortcoming that no German, not to say Bavarian, material was revised and that only one specimen from Switzerland was studied. As was the case with the 2004 volume, the conspicuous lack of material from northern and eastern Europe may raise questions about the accuracy of some species concepts employed in this work as well as for some claims of novelty.

This work is divided into three parts of which the first two ("Introduction" and "Matériel et méthodes") are quite brief and refer the reader to the 2004 volume. The remainder of the work is dedicated to "Présentation des espèces," which is divided into four parts: (A.) "*Amanita pini*" (a slight modification of the protolog of this taxon), (B.) "Espèces nouvelles," (C.) "Quelques *Vaginatinae* déja décrites, liées a un hôte précis," and (D.) "Les Amanites cocardées européennes." There are 26 line drawings of anatomical elements, and thirty-six photographic plates in color were included at the end of the book. Overly dark photographs and photographs that are off color were not adjusted prior to publication. The book lacks an index, but does list the taxa that are treated at greatest length in the table of contents. The authors do not follow conventions for presenting their lists of material examined in a manner organized by countries and by administrative subdivisions of those countries; as a consequence, their information on material examined is very difficult to read. The present work contains nine new names and one new combination in *Amanita*. Six previously named taxa are treated with some detail. Three names have lectotypes designated, and one name has a neotype designated. The new taxa are proposed to be added to the 50 or so that the authors estimate have been previously treated in Europe, based on certain keys authored by M. Contu. These keys contain a significant number of infraspecific taxa and several synonyms; hence, the species count in them is closer to 30.

The new species proposed herein (each listed with the country or countries from which material examined was collected) are *A. betulae* (France), *A. coryli* (France and Spain), *A. fulvoides* (France, Italy, and Spain), *A. schaefferi* (a single locality in France), and *A. subfuliginosa* (a single Italian locality). *Amanita avellanea* is the new combination at species rank. The authors' *A. ochraceomaculata* (which is extremely close to, if not identical with, *A. fulva* as it is widely understood in northern Europe) is presented again without comparison to any truly similar taxa. Among other previously described taxa treated in part C are *A. pachyvolvata* and *A. simulans*. *Amanita umbrinolutea* and *A. battarrae* are treated in part D.

The authors unfortunately follow the approach of the 2004 volume and abandon both the use of logic and the treatment of complex and informative morphological characters such as the detailed anatomy of the lamellae. In fact, I was unable to find a species treatment in the book that mentions bilateral

divergent lamella trama. Demonstrations of novelty are inadequate and consider only European taxa that bear some macroscopic similarity with a given proposed taxon. The authors take little advantage of, and make little comparison to, extant expert literature. In one case, a modern revision of a taxon that they treat at length (*A. umbrinolutea*) is included in their bibliography but cited briefly with regard to a nomenclatural point and is ignored entirely with regard to its taxonomic content — even though the authors' description differs significantly from that of the prior work. When a prior work treats more characters than do the authors, the authors select from the prior work only the information that may be placed in one-to-one comparison with components of their own description and omit mention of the rest. From the work of Bas in section *Vaginatae* they cite only his paper on species concepts (Bas 1977). Sixteen years of relevant publications by Yang and his co-authors are ignored entirely. In some critical cases, the authors make unjustified assumptions about the meaning of the work of other amanitologists such as those just cited. For example, the authors assume that because they have noted that the cells of the subhymenium in many species of *Amanita* inflate to one degree or another as the hymenium matures, that the researchers who describe the subhymenium and characters of the subhymenium in the development of *Amanita* taxonomy must be unaware of this fact and, hence, have been producing ill-informed, wrong descriptions. As a consequence of the above-cited issues (among other problems) the descriptions in the reviewed work are very difficult, if not impossible, to compare with the descriptions that comprise the main body of morphological study of the *Amanitaceae* over the last half century.

Spore data in this work cannot be understood by other workers [unless they were to have access to the original complete sets of raw spore measurement data] because, despite including the table from (Bas 1969) relating length/breadth ratio (Q) ranges to qualitative terms for spore shape, the authors almost always provide ranges for Q that include values less than 1.0. Hence, their average Q values are meaningless.

Thorough type revisions will be necessary in order to give meaning to every name proposed in this work. All type collections are retained in personal herbaria, as was often the practice in the case of taxa treated in Neville and Poumarat (2004). I have inquired of Prof. Poumarat concerning the location of materials revised for the present work as well as the 2004 volume. He kindly informed me that all the materials of Neville's herbarium have now been transferred to Prof. Poumarat's herbarium, and he is considering where to deposit them in the future. Great effort has been expended on assembling and determining the material in the combined herbaria of Neville and Poumarat; it will be important that the material be well preserved and to know where the exsiccata can be found.

The authors' reasoning in attempting to replace the use of anatomy with supposed single-tree-genus associations in the diagnoses of their species is circular. It only means that someone other than the authors must do the anatomical revision to support the species and create a sufficiently detailed species concept so that hypotheses concerning plant association, for example, can become meaningful.

Poor methodology and lack of anatomical character data lead to difficult situations of which there is a prime example in this book. "Species A" is defined by a limited set of anatomical details and the fact that "species A" is associated with a certain tree genus or genera, let's call the associates collectively "T." It happens that many examples of "species A" have been collected over a number of years around a single specimen belonging to the T group. It is assumed that all the specimens (being macroscopically similar and, apparently, sharing the limited set of microscopic characters used) are all one species. Moreover, it is assumed without justification that all the basidiomes are produced by a single mycelium. One set of the few available anatomical characters (spore size and shape) varies considerably from one collection to another of "species A." A chart is offered showing a distribution of the average length/width ratio (the quantitative rendering of "shape") that is far from a normal distribution and strongly suggests to me the presence of more than one taxon in the set of collections. Because the anatomical information has been intentionally limited in favor of ecological association information and because of the cited unjustified assumptions related to ecological association, the possibility that multiple species may be involved is not considered.

This book is nomenclaturally significant. It also brings to light opinion and information from some southern European publications that are not easily accessible elsewhere. It should be in libraries for the use of those interested in revising the taxa discussed and proposed in it.

Bas C. 1969. Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. Persoonia 5: 285–579.

Bas C. 1977 ["1976"]. Species concept in *Amanita* section *Vaginatae in* H. Clémençon (ed). The species concept in *Hymenomycetes*. Bibliotheca Mycologica 61: 79–103.

Corner EJH, Bas C. 1962. The genus *Amanita* in Singapore and Malaya. Persoonia 2: 241–304.

Neville P, Poumarat S. 2004. *Amaniteae*. 1. Fungi Europaei 9. Edizione Candusso.

Yang ZL. 1997. Die *Amanita*-Arten von Südwestchina. Bibliotheca Mycologica 170: i–ii, 1–240.

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Stereoid fungi of America. By L. Ryvarden, 2010. Synopsis Fungorum, P.O.Box 95, Blindern, 0314 Oslo, Norway. <leif.ryvarden@bio.uio.no>. Pp. 202, figs 65. [Synopsis Fungorum vol. 28] Price: NOK 280.

***Stereum* s.l.** By A.L. Welden, 2010. New York Botanical Garden Press, Bronx, New York, NY 10458-5126, USA; <www.nybgpress.org>. Pp. 79, figs. [Flora Neotropica Monograph 106] ISBN 0893275042. Price: $ 38.00.

In the previous installment of this journal's Bookreviews (Mycotaxon 114: 490), David Hawksworth, in noting the paucity of identification keys in a recent collection of systematic revisions of *Dothideomycetes*, stated: "Mycologists with access to superbly equipped and resourced molecular laboratories, supported by skilled technicians, should not forget that they represent a privileged section of the potential user-community of systematic works." If strictly phylogenetic studies lacking morphological information (noting that not all of the chapters in the aforementioned collection fit this description) represent one end of a "utility vs. phylogenetic purity" spectrum, the other must consist of studies that eschew monophyly entirely to facilitate identification of superficially similar taxa. Two recent treatments of stereoid fungi of the Americas inhabit the latter niche.

In thinking of the stereoid fungi (*Stereum* s.l.), one may be reminded of former U.S. Supreme Court Justice Potter Stewart's famous statement about pornography: "I know it when I see it." Beyond this initial recognition, however, the task of identification is considerably more complicated. The characters uniting the stereoid fungi are quite general ones — effused-reflexed or pileate basidiomata with a smooth or nearly smooth hymenophore and hyaline, usually smooth, basidiospores — and further examination reveals a significant amount of anatomical and micromorphological diversity. Given the dearth of accessible sources for identification of stereoid fungi, a central starting point for taxonomic navigation seems justified. The two volumes reviewed here provide such resources, and in so doing take a decidedly non-phylogenetic approach: a total of 37 genera representing 18 families and 6 orders (according to current Index Fungorum taxonomy) are covered.

The volume by Arthur L. Welden (Tulane University, emeritus), the 106th in the *Flora Neotropica* series of monographs, covers 13 stereoid genera having species in the Neotropics. The author provides a detailed methods section (including formulae for all macrochemical reagents employed in the study), a discussion of the phylogenetic affinity of each genus, a synoptic definition of *Stereum* s.l. (the author employs this definition with some skepticism, but it is nonetheless welcome to the reader in defining the limits of the entity to be discussed), a key to genera, keys to Neotropical species of *Coralloderma*, *Stereopsis*, *Cotylidia*, *Hydnopolyporus*, *Cymatoderma*, *Podoscypha*, *Cystostereum*,

Stereum, and *Lopharia* s.l., and descriptions of species. The volume is illustrated by 13 line drawings and 6 distribution maps; the former generally include macroscopic habit as well as micromorphological sketches. The section on phylogenetic affinities includes an extended discussion of *Cymatoderma* and *Podoscypha* (together accounting for just over half of the known Neotropical *Stereum* s.l.), including detailed discussions of major characters and a tabular species-level comparison of character state distributions. Taxonomic novelties presented in this volume include 7 new combinations and one status reassignment. This handsome and carefully prepared volume reflects a very high level of scholarship, clearly drawn from both extensive fieldwork and a thorough knowledge of the literature regarding stereoid fungi.

The volume by Leif Ryvarden (University of Oslo) is intended to be a compilation of available information to aid in the identification of stereoid fungi — especially in the Neotropics — rather than a monographic study, though one new species and 2 new combinations are proposed. Thirty-six genera are included. The larger number of genera compared to the Welden volume is a function of broader geographic and taxonomic coverage (temperate as well as tropical America, and taxa with ornamented and smooth spores, are included); in other cases it is due to differences in delimitation of genera — for example, *Hjortstamia* and *Porostereum* are retained within a more heterogeneous *Lopharia*, *Aleurocystis magnispora* is treated in *Stereum*, and *Cyphellostereum pusiolum* is treated within *Cotylidia* by Welden. The text provides summary information for each genus (original citation, brief descriptive synopsis, type species data, and remarks) and descriptions of each of the treated species, and is accompanied by a key to genera (albeit incomplete; see below), 22 keys to species, and 75 line drawings of micromorphological features and in some cases basidiome habit. Some of the illustrations are provided by the author; others are reprinted from *Corticiaceae of North Europe*. There are several typographic errors, including a misspelling of *Licrostroma* in the table of contents, discrepancy between the number of included genera on the back cover (35) vs. table of contents (36), and reprinted figures that retain internal references to additional figures from the original text. The first printing lacked the second part (Key B) of the key to genera, but this problem was corrected in later printings. Despite a few such errors, this is an excellent volume that compiles an impressive amount of information, and the identification tools and remarks presented here will prove valuable to not only those mycologists working in the Americas but on stereoid fungi in other parts of the world as well.

Both Welden and Ryvarden have opted to present polyphyletic assemblages of taxa in hopes of providing mycologists in tropical America as well as students and non-specialists with a central starting point from which to identify a wide variety of these superficially similar fungi. While this approach

is completely justified given the need for accessible, comprehensive, and up-to-date taxonomic literature, its ability to provide a catalyst for further, detailed scientific study of particular monophyletic subgroups is diminished by the lack of a clear phylogenetic context. Welden's discussion on "The interrelationships of the genera of *Stereum* s.l." bridges this gap to a large degree, but the interested researcher will need to consult a wider variety of sources in order to understand the broader evolutionary picture for these organisms. Assuming that mycologists with access to molecular laboratory facilities also have access to suitable electronic reference materials, and given the greater ability for electronic databases such as Index Fungorum and Mycobank to keep up with rapidly changing systematic classifications compared to print sources, the approach taken by both authors seems to be the right one. Both of the volumes reviewed here will be valuable references for workers with an interest in further sorting out taxonomic issues within the stereoid fungi and/or identifying stereoid fungi in the Americas.

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Ferrugens: Diversidade de *Uredinales* do Parque Nacional do Itatiaia, Brasil.
By M. Salazar Yepes & A. Alves de Carvalho Júnior. 2010. Technical Books Eidtora, Rua Gonçalves Dias, 89, 2° andar, Sala 208, Centro Rio de Janeiro RJ CEP 20.050-030, Brazil. <www.tbeditora.com.br>. Pp. 201, figs 36. ISBN: 978-85-61368-18-0. Price: R$ 70.00.

The Parque Nacional do Itatiaia is the oldest national park in Brazil, and part of the Atlantic forest, well known for its high biodiversity. It is in the provinces of Rio de Janeiro and Minas Gerais, and covers the higher altitudes (above 500 m asl). This is the area where the two authors have inventoried the rust fungi. Almost one third of all the species known for Brazil so far (Hennen et al. 2005) is present in the park, 171 species in total. The book starts with short introductions to the park and its vegetation and to rust fungi, followed by the main part with the descriptions of the species organized by host plant, *Pteridophyta* first, then the *Magnoliaphyta* with all their families. Colour photos of infected plants and microscope photos of spores and other important features illustrate the fungi. As expected, the genus *Puccinia* is the most species rich with 81 species (this excludes *Uromyces* with 26 species). The *Asteraceae* are the plant family with the highest number of rust fungi. The book continues with a glossary, a bibliography, an index to the rust species, and per plant species a list of rust taxa. The different chapters are clearly colour coded on the pages, making it very easy to find the different subjects.

This is the first book of this kind that I have ever seen with colour illustrations throughout. It is a well-executed, important contribution to the knowledge of those fascinating plant pathogenic fungi. I would have loved to see more, and bigger pictures of the infected plants, and family names as page headers, but these are just two minor comments. Even though it is written in Portuguese, the text and descriptions are quite accessible and understandable. All in all a very nice, well-executed contribution to our knowledge of an important fungal group.

Hennen JF, Figueiredo MB, de Carvalho AA Jr, Hennen PG, 2005. Catalogue of the species of plant rust fungi (*Uredinales*) of Brazil. 490 pp. <www.jbrj.gov.br/>

Checklist

List of fungi recorded in Japan. By Ken Katumoto. 2010. The Kanto Branch of the Mycological Society of Japan. (Contact: Toru Okuda <torula@lab.tamagawa.ac.jp>.) North American distributor: Mycotaxon Ltd., P.O. Box 264, Ithaca, NY 14851-0264, USA. <info@mycotaxon.com>. Pp. xv + 1177. Softcover ed. ISBN 978-4-87974-624-5. Price (US) $130, postpaid. Hardcover ed. ISBN 978-4-87974-625-2. Price (US) $220, postpaid. Canada and Mexico delivery add US$25 for postage.

Access to this monumental work is essential for any taxonomist who works with collections from Japan. It is indisputably the most comprehensive floristic listing that I have ever encountered. 1067 pages are, throughout, in small typeface, alphabetically listing all species by alphabetically ordered generic name, with the most current familial placement shown for each taxon. The correct names and authors are all provided with their places of publication in Roman typeface. Locations of holotypes and isotypes are noted carefully. Anamorphs where known are clearly identified, allowing quick cross-referencing. Teleomorphs are similarly cross- referenced to their anamorph names. Nomina rejicienda under the International Code of Botanical Nomenclature are clearly marked as such. Even more important, all misapplications are shown as "sensu" a Japanese author or authors, and crossed referenced to those names. All in all, a goldmine of information.

The volume weighs in at almost 4 1/2 pounds in paperback and over 5 pounds in hardcover, and may to some of us be worth its weight in gold. Though few individuals will be willing to pay the price for a personal copy, the serious taxonomists among us should do all they can to get their library to buy a copy. Borrowing a copy on interlibrary loan is scarcely the solution when time and time again you'll want to consult this.

With all its excellent features, this book has some drawbacks for the non-Japanese reader. Host names are given by Japanese common name only, and these in kanji. Common names in kanji are also applied to most accepted

species for Japan. For non-kanji readers, the two indices to Japanese names of fungi and Japanese names of hosts will prove useless. And, to my mind, the most serious fault for the non-kanji reader is that later references to each species are provided, but only non-Japanese journals have these references in Roman typeface. All Japanese journal names, except for original publication of a name, are strangely rendered only in kanji. One sees three, four, five or more kanji characters followed by, for example, 26:65, 1975., leaving the non-Japanese reader wondering which journal that is. I'm sure our Japanese colleagues will be frequently emailed to translate such journal titles into Roman characters. I have suggested to the publishers that they could add a helpful slip-in sheet with the translations of the names of Japanese journals into Roman typeface.

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Book Announcements

Atlas des Cortinaires Vol. 19. By A. Bidaud, P. Moënne-Loccoz, P. Reumaux et X. Carteret. 2010. Sarl Editions FMDS, 2436 route de Brailles, 38510 Vézeronce-Curtin, France. <esperance.bidaud@wanadoo.fr>. Loose leafed in folder. Pp. 131, pl. 64. Price around € 148.00.

Flora Slovenska Volume 10:2: *Mycota* (Huby), *Ascomycota* (Vreckatem huby). *Taphrinales: Protomycetaceae, Taphrinaceae.* 2010. Edícia Flóra Slovenska, Slovakia. <www.elegenda.sk>. Pp. 184, pl. 6, figs. ISBN: 978-80-224-1096-0. Price € 11.00.

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Regional annotated mycobiotas new to www.mycotaxon.com

MYCOTAXON is pleased to announce the addition of six new species distribution lists to our "web-list" page at www.mycotaxon.com//resources/weblists.html, where over 70 checklist PDFs are available for free download. The content and design of each list is the sole responsibility of its authors and their three (or more) expert reviewers, after which those meeting MYCOTAXON's scientific and nomenclatural criteria are accepted for posting. The authors, titles, and abstracts of our most recently accepted web-lists are provided below.

CENTRAL AMERICA

Nicaragua

Delgado, Gregorio. Nicaraguan fungi: a checklist of hyphomycetes. 31 pp.

ABSTRACT— A checklist of hyphomycetes (anamorphic fungi) known from Nicaragua is presented. A total of 194 taxa belonging to 70 genera are listed, including 25 determined only to generic level, 156 to specific level and 13 infraspecific taxa. They were compiled from scattered records in the literature, the online databases of the world's major culture collections and fungal herbaria, and sporadic collections of plant debris samples carried out in the departments of Managua and León in 2008. 14 saprobic genera, 19 species and one forma are recorded for the first time from Nicaragua. A host and substrate index is also provided, including 99 host plants belonging to 30 families and 16 host insects belonging to 4 orders. A brief approach to the history of mycology in the country is presented.

Panama

Piepenbring, Meike, José Camarena, Dario Cruz, Ana Karina Gómez, Yuriza Guerrero, Tina Antje Hofmann, Roland Kirschner, Mavis de Matas, Loraine Perez, Délfida Rodríguez, José Ureta, Ivette Vargas & Carl Williams. New records of fungi pathogenic on cultivated plants in Panama. 11 pp.

ABSTRACT— Knowledge about the geographical distribution of species of plant pathogenic fungi is fundamental for epidemiology, decision making and recommendations for their control. For many tropical countries like Panama, however, checklists are still very incomplete. In the present publication, 20 species of pathogenic fungi on cultivated plants are cited, with 13 of them and one hyperparasitic fungus reported for the first time for Panama, mainly in the province of Chiriquí in the western part of the country. Among these are several rarely reported species:

Asterinella puiggarii on *Psidium guajava; Entyloma doebbeleri* on *Dahlia* sp., which is reported for the first time outside Costa Rica; *Mycosphaerella agapanthi-umbellati* on *Agapanthus* sp., which is recorded for the first time for America; *Passalora vanderystii* on *Phaseolus vulgaris*; and *Ustilago affinis* on *Stenotaphrum secundatum*.

South America

Brazil

Gumboski, Emerson Luiz & Sionara Eliasaro. Checklist of lichenized fungi of Santa Catarina State (Brazil). 45 pp.

Abstract— Based on the evaluation of available literature, a list of 355 lichenized fungi species recorded from Santa Catarina State, Brazil is presented. These species are distributed among 109 genera and 45 families. *Parmeliaceae* and *Cladoniaceae* are the most diverse families with 69 and 41 species, respectively.

Europe

Greece

Polemis, Elias, Dimitris M. Dimou, Leonidas Pountzas, Dimitris Tzanoudakis & Georgios I. Zervakis. Mycodiversity studies in selected ecosystems of Greece: 5. Basidiomycetes associated with woods dominated by *Castanea sativa* (Nafpactia Mts., central Greece). 16 pp.

Abstract — Very scarce literature data are available on the macrofungi associated with sweet chestnut trees (*Castanea sativa, Fagaceae*). We report here the results of an inventory of basidiomycetes, which was undertaken in the region of Nafpactia Mts., central Greece. The investigated area, with woods dominated by *C. sativa*, was examined for the first time in respect to its mycodiversity. One hundred and four species belonging in 54 genera were recorded. Fifteen species (*Conocybe pseudocrispa, Entoloma nitens, Lactarius glaucescens, Lichenomphalia velutina, Parasola schroeteri, Pholiotina coprophila, Russula alutacea, R. azurea, R. pseudoaeruginea, R. pungens, R. vitellina, Sarcodon glaucopus, Tomentella badia, T. fibrosa* and *Tubulicrinis sororius*) are reported for the first time from Greece. In addition, 33 species constitute new habitats/hosts/substrates records.

Italy

Rizzi, Guido, Guido Incerti, Fabrizio Ginaldi, Danijela Kodnik, Serena Viglione & Paolo Giordani. A contribution to the lichen flora of Sardinia. 27 pages.

Abstract A contribution to the epilithic and epiphytic lichen flora of Sardinia is presented. Seventy localities from both coastal and mountain areas of western Sardinia have been investigated during four surveys from 2006 to 2008. In all, 390 taxa have been recorded. Three taxa are new to Italy (*Allarthonia hypobela, Bacidia biatorina, Catillaria subviridis*); 25 taxa are new to Sardinia. *Pannaria rubiginosa* and *Melaspilea ochrothalamia* were re-collected after one century. Distribution and substrata are presented.

MID-EAST

Turkey

Tufan-Çetin, Özge & Hüseyin Sümbül. Lichens of the Köprülü Canyon National Park in Turkey. 25 pp.

ABSTRACT — This is the first comprehensive survey on lichens of the Köprülü Canyon National Park. Totally 1266 lichen samples were collected during the field studies between June 2006 and August 2008 in Köprülü Canyon National Park. 217 lichen taxa which belong to 8 orders, 28 families and 76 genera were determined from the research area. Of the determined lichens 203 taxa were recorded for the first time in the national park. In addition 67 taxa are new records for Antalya and 7 taxa for Turkey.

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bad taxonomy

can KILL